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SELECTION OF ELECTROPHORIC DERIVATIVES OF 1-AMINOPYRENE AND 2-AMINOFLUORENE FOR DETERMINATION BY GAS CHROMATOGRAPHY WITH ELECTRON-CAPTURE NEGATIVE-ION MASS SPECTROMETRY

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SUMMARY

Several electrophoric derivatives of 1-aminopyrene and 2-aminofluorene were prepared. Reagents such as heptafluorobutyryl chloride, pentafluorobenzoyl chloride, pentafluorobenzyl bromide and pentafluorobenzaldehyde, alone and in certain combinations, were employed. The ease of formation, yield, stability and fragmentation by gas chromatography with electron-capture negative-ion (ECNI) mass spectrometry of the derivatives were compared. This allowed the most promising ones to be selected for future work on the sensitive detection of aminopolyaromatics by this detection technique. Pentafluorobenzylidene (first choice) and N-pentafluorobenzyl-N-heptafluorobutyryl (second choice) derivatives emerged as the best ones. The origins of losses of HF and 2HF from some of the derivatives were elucidated in the ECNI mass spectra by studies of deuterium-labeled analogues.

INTRODUCTION

Nitrated polycyclic aromatic hydrocarbons (NO_2-PAHs) are common environmental pollutants. Since several NO_2-PAHs are potent mutagens in the Ames assay and tumorigenic in animal bioassays[1–3], the measurement of this class of com-

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pounds is of interest. Analyses of environmental extracts for NO_2-PAHs have been performed using high-performance liquid chromatography (HPLC) and gas chromatography (GC) with a variety of selective detectors, including electron-capture[4], nitrogen–phosphorus[5] and electrochemical[6]. Perhaps the most useful approach, however, has been GC combined with electron-capture negative-ion mass spectrometry (ECNI-MS)[7–13]. This technique provides the high sensitivity and selectivity necessary at low sample concentrations. Since NO_2-PAHs have moderate to good electron capture properties, they are suited to this type of analysis. Korfmacher and Rushing[8] have reported detection limits in the 1–5 pg range for several mono- and dinitro-PAHs. Nevertheless, because of high levels of interfering compounds present in the enrivonmental samples and the significant range in electron-capture response for NO_2-PAHs[14], others have reduced the nitro group to an amine and then derivatized the latter with a perfluoroalkyl anhydride in order to provide a more specific or sensitive analysis[4,15,16]. Earlier work on the toxicology and chemical analysis of NO_2-PAH has been reviewed[14].

Nitro-PAHs are metabolized in mammals by oxidative and reductive pathways, or by a combination of both[17]. A major metabolic pathway leading to DNA adduct formation is through reduction of the nitro group to an N-hydroxyamino derivative. Since subsequent reduction leads to an amino-PAH, the extent of formation of this latter metabolite should be an indication of the degree to which the nitro-PAH underwent activation by this pathway. Amino derivatives of dinitro-PAHs have been analyzed by ECNI-MS[12]; however, amino-PAHs typically do not chromatograph well on GC columns and, unless a nitro function is retained, they are not very sensitive by ECNI-MS. It is therefore appropriate to convert them to suitable electrophoric derivatives. Recently Trainor and Vouros[18] described the ECNI-MS properties of electrophore-derivatized chloroanilines.

In this paper we examine several electrophoric derivatives of the two NO_2-PAH compounds, 1-aminopyrene (1-AP) and 2-aminofluorene (2-AF). Ease of formation, yield, stability and GC–ECNI-MS properties of the products were the criteria applied in order to select the best derivative.

EXPERIMENTAL

Chemicals

2-Nitrofluorene (2-NF), 1-nitropyrene (1-NP), 1-AP, 2-AF, heptafluorobutyryl chloride (98%), heptafluorobutyric anhydride (98%) and methyl iodide were purchased from Aldrich (Milwaukee, WI, U.S.A.). 1-AP and 2-AF were purified by flash column chromatogrpahy using Silica gel 60 (230–400 mesh, EM Science, Cherry Hill, NJ, U.S.A.) with ethyl acetate–hexane (60:40, v/v) and then recrystallized using ethyl acetate–hexane (40:60). Pentafluorobenzyl bromide and pentafluorobenzoyl chloride were obtained from Columbia Organic Chemicals (Camden, SC, U.S.A.). [$^2H_{10}$]Pyrene (98%), [$^2H_{10}$]fluorene (98%) and [$^2H_3$]methyl iodide were purchased from Cambridge Isotope Labs. (Woburn, MA, U.S.A.). Nitrogen dioxide gas was from Matheson (Gloucester, MA, U.S.A.). All solvents were HPLC grade.

Instrumentation

The GC–MS measurements were obtained on a Finnigan 4021B mass spectrometer interfaced to a Hewlett-Packard 5890A gas chromatograph. A Hewlett-

Packard 5% phenyl methyl silicone capillary column, 17 m × 0.32 mm (0.17 μm film thickness), was inserted directly into the ion source. On-column injections were made and the GC column was programmed from 70 to 300°C at 30° min^{-1}. The source temperature was held at 200°C. Helium (10 p.s.i.) and methane (0.25 Torr) were used, respectively, as the carrier and ECNI buffer gases. Both gases were purified through oxygen scrubber and moisture traps (Alltech, Deerfield, IL, U.S.A.). Electron-impact (EI) spectra (70 eV, 50mA) were recorded by GC–MS with scanning from 40 to 600 a.m.u. in 0.95 s. Positive-ion chemical ionization and ECNI spectra were similarly recorded. Selected-ion monitoring (SIM) experiments were conducted by scanning each mass for 0.4 s.

Accurate mass measurements were performed under EI conditions (70 eV) on a VG70-250SE (VG Instruments, Stamford, CT, U.S.A.) forward geometry double-focusing mass spectrometer. Samples were introduced via a direct insertion probe.

^1H NMR spectra were recorded with a Varian XL-300 spectrophotometer (Varian Instruments, Walnut Creek, CA, USA) with tetramethylsilane as the internal standard. Thin-layer chromatography (TLC) with silica gel GF was performed with Uniplates (Analtech, Newark, DE, U.S.A.) containing a fluorescent indicator: 250-μm plates were used for monitoring reactions and 1000-μm plates were used for preparative TLC. The glassware used for HPLC and GC was acid-washed and gas phase-silanized.

HPLC was performed on a Waters 600 system, equipped with a U6K injector and a 20-μl sample loop. An Alltech Uptight C_8 silica (17.5 cm × 0.46 cm, 5-μm particles) analytical column was preceded by a precolumn (4.5 cm x 0.46 cm, Upchurch Scientific, Oak Harbor, WA, U.S.A.) packed with Vydac C_{18} material (Separations Group, Hesperia, CA, U.S.A.). The detector was a Waters (Milford, MA, U.S.A.) Model 481 Lambda-Max which was interfaced to a LDC/Milton Roy (Riviera Beach, FL, U.S.A.) CI-10 recorder. The mobile phase was methanol–water (85:15, v/v) at a flow-rate of 1 ml/min.

GC–ECD was carried out on a Varian 3700 instrument equipped with a ^{63}Ni electron-capture detector. A Hewlett-Packard (Andover, MA, U.S.A.) 17 m x 0.32 mm 5% phenyl methyl silicone capillary column (0.17 μm film thickness) was used. Injections were made in the on-column mode. The oven was programmed from 80 to 240°C at 40°C/min with a 2-min initial hold time. The injector was programmed from 50 to 240°C at 180°C/min. The detector temperature was set to 340°C. Helium (5 ml/min) and nitrogen (25 ml/min) were used as the carrier and make-up gases, respectively.

All solution compositions were v/v unless indicated otherwise.

Synthesis

*N-Heptafluorobutyryl-1-aminopyrene (**1**).* To a solution of 1-AP (217 mg, 1 mmol) in acetonitrile (2.5 ml) under nitrogen was slowly added heptafluorobutyric anhydride (820 mg, 2 mmol). The mixture was stirred at room temperature for 30 min and then evaporated to dryness *in vacuo*. The residue was washed twice with hexane and recrystallized from ethanol, giving 339 mg (82%) of product. ^1H NMR ([$^2H_6$]dimethylsulfoxide, [$^2H_6$]DMSO), δ 8.06–8.18 (m, 3 H, ArH), 8.27–8.41 (m, 6 H, ArH), 12.00 (br s, 1 H, NH); IR (KBr) 1200, 1500, 1690, 3260 cm^{-1}; MS (70 eV), *m/z* (relative intensity) 413 (M^+, 100), 216 (51), 214 (16), 189 (31).

*N-Heptafluorobutyryl-N-methyl-1-aminopyrene (**2**).* To a mixture of **1** (103 mg, 0.25 mmol) and potassium carbonate (690 mg, 1.25 mmol) in acetone (2 ml) was added methyl iodide (1.42 g, 2.5 mmol). The mixture was stirred at room temperature for 24 h. After filtration, the filtrate was evaporated and the residue was recrystallized from 60% ethyl acetate in hexane, giving 91 mg (85%). ^{1}H NMR (C^2HCl_3) δ 3.55 (s, 1 H, CH_3), 7.84–8.32 (m, 9 H, ArH); IR (KBr) 1200, 1675; MS (70 eV), *m/z* 427 (M^+, 80), 230 (18), 218 (30), 217 (32), 210 (100), 202 (50).

*N-Heptafluorobutyryl-N-pentafluorobenzyl-1-aminopyrene (**3**).* Compound **1** was alkylated with pentafluorobenzyl bromide as described for **2** and recrystallized from ethyl acetate–hexane (60:40) to yield 89 mg (60%) of product. ^{1}H NMR (CF_3COOH) δ 4.78 (s, 2 H, CH_2), 8.08–8.60 (m, 9 H, ArH); IR (KBr) 1150, 1470, 1640; MS (70 eV), *m/z* 593 (M^+, 22), 412 (14), 394 (18), 243 (100), 216 (45), 214 (45), 189 (25), 181 (32).

*N-Pentafluorobenzoyl-1-aminopyrene (**4**).* To a solution of 1-AP (434 mg, 2 mmol) in 5 ml of acetonitrile was added pentafluorobenzoyl chloride (922 mg, 4 mmol) and the mixture was stirred at room temperature for 30 min. After filtration and washing twice with hexane, the precipitate was heated with 50 ml methanol. The insoluble material was filtered off from the hot solution. Concentration and cooling of the methanolic solution gave 329 mg (40%) of product as pale green needles. ^{1}H NMR ([$^2H_6$]DMSO) δ 8.13–8.37 (m, 9 H, ArH), 11.40 (br s, 1 H, NH); IR (KBr) 1475, 1665, 3320; MS (70 eV), *m/z* 411 (M^+, 65), 243 (100), 216 (45), 214 (60), 189 (38).

*N-Methyl-N-pentafluorobenzoyl-1-aminopyrene (**5**).* To a mixture of **4** (103 mg, 0.25 mmol) and potassium carbonate (690 mg, 1.25 mmol) in dimethylformamide (2 ml) was added methyl iodide (1.40 g, 2.5 mmol). The mixture was stirred at room temperature for 24 h. After filtration and evaporation of the solvent, the residue was recrystallized from 60% ethyl acetate in hexane to give 80 mg (75%) of product. ^{1}H NMR (C^2HCl_3) δ 3.74 (s, 3 H, CH_3), 7.78–8.28 (m, 9 H, ArH); IR (KBr) 1480, 1640, 2900; MS (70 eV), *m/z* 425 (M^+, 55), 230 (12), 208 (100), 202 (28), 195 (14).

*N-Pentafluorobenzyl-1-aminopyrene (**6**) and N,N-bis-pentafluorobenzyl-1-aminopyrene (**7**).* To a solution of 1-AP (217 mg, 1 mmol) in acetonitrile (5 ml) was added potassium carbonate (140 mg, 1 mmol) and pentafluorobenzyl bromide (260 mg, 1 mmol). The mixture was stirred at 60°C for 16 h. The solid was filtered off from the reaction mixture and the filtrate was concentrated. Products **6** and **7** were separated by preparative TLC with ethyl acetate–hexane (20:80) as the eluent. Product **6**: yield 60 mg (15%); ^{1}H NMR ([$^2H_6$]DMSO–CF_3COOH, 6:1) δ 3.95 (s, 2 H, CH_2), 7.35 8.05 (m, 9 H, ArH), 9.30 (S, 1 H, NH); IR (KBr) 1490, 3380; MS (70 eV), *m/z* 397 (M^+, 62), 216 (100), 189 (52), 181 (18). Product **7**: yield 115 mg (20%); ^{1}H NMR ([$^2H_6$]DMSO) δ 4.50–4.70 (br s, 4 H, CH_2), 7.86–8.24 (m, 9 H, ArH); IR (KBr) 1485, 2820, 2880; MS (70 eV), *m/z* 577 (M^+, 49), 396 (100), 228 (28), 217 (60).

*N-Pentafluorobenzylidenyl-1-aminopyrene (**8**).* To a solution of 1-AP (217 mg, 1 mmol) in acetonitrile (5 ml) was added pentafluorobenzaldehyde (980 mg, 5 mmol). The mixture was stirred at room temperature for 10 min. The product was filtered and washed with hexane. Recrystallization from ethyl acetate gave 360 mg (92%). ^{1}H NMR ($C_6{}^2H_6$–[$^2H_6$]DMSO, 6:1) δ 8.18–8.45 (m, 9 H, ArH), 10.24 (s, 1 H, N=CH); IR (KBr) 1600; MS (70 eV), *m/z* 395 (M^+, 100), 201 (75), 101 (54).

*N-Heptafluorobuturyl-2-aminofluorene (**9**).* This compound was prepared from

2-AF as described for **1**. The residue was washed twice with hexane and recrystallized from ethanol. Yield: 287 mg (76%); ^{1}H NMR ([$^2H_6$]DMSO) δ 3.95 (s, 2 H, CH_2), 7.31–7.39 (m, 2 H, ArH), 7.57–7.68 (m, 2 H, ArH), 7.87–7.96 (m, 3 H, ArH), 11.36 (s, 1 H, NH); IR (KBr) 1190, 1690, 3330; MS (70 eV), *m/z* 377 (M^+, 100), 180 (30).

*N-Heptafluorobutyryl-N-methyl-2-aminofluorene (**10**).* This compound was prepared from compound **9** as described for **2**. Yield: 86 mg (86%); ^{1}H NMR ([$^2H_6$]DMSO) δ 3.38 (s, 3 H, CH_3), 3.95 (s, 2 H, CH_2), 7.36–7.97 (m, 7 H, ArH); IR (KBr) 1210, 1665; MS (70 eV), *m/z* 391 (M^+, 100), 210 (30), 165 (40).

*N-Heptafluorobutyryl-N-pentafluorobenzyl-2-aminofluorene (**11**).* Compound **9** was alkylated with pentafluorobenzyl bromide as described for **2** to give 120 mg of **11** (86%). ^{1}H NMR (C^2HCl_3) δ 4.00 (s, 1 H, CH_2), 5.19–5.30 (br d, 2 H, N–CH_2), 7.18–7.91 (m, 7 H, ArH); IR (KBr) 1220, 1495, 1685; MS (70 eV), *m/z* 557 (M^+, 38), 376 (21), 207 (100), 181 (35), 165 (25).

*N-Pentafluorobenzylidenyl-2-aminofluorene (**12**).* This compound was prepared from 2-AF as described for **8**. Yield: 309 mg (86%); ^{1}H NMR (C^2HCl_3) δ 3.96 (s, 1, H, CH_2), 7.32–7.85 (m, 7 H, ArH), 8.67 (s, 1 H, N–CH); IR (KBr) 1480; MS (70 eV), *m/z* 359 (M^+, 24), 340 (100).

*[$^2H_9$]-1-Nitropyrene (**13**).* This compound was prepared by the technique of Radnar[19]. To a solution of [$^2H_{10}$]pyrene (26 mg, 0.01 mmol in 10 ml of dichloromethane) were added 750 μl of 0.1 *M* N_2O_4 in dichloromethane. The mixture was stirred at room temperature and monitored by HPLC (acetonitrile–water, 53:47) and TLC (toluene–cyclohexane, 20:80). Formation of a single product was observed at the end of the reaction (20 min). After the evaporation of the solvent, the residue was washed with water and residual water was removed as an azeotrope with acetonitrile. MS (70 eV), *m/z* 266 (M^+).

*[$^2H_9$]-1-Aminopyrene (**14**).* The residue from above was combined with 60 mg of zinc dust, 40 ml of methanol and 4 ml of 5% ammonium chloride in water and stirred at room temperature[20]. The reaction was monitored by TLC (acetone–hexane, 20:80) and HPLC. After 1 h the mixture was filtered. Water (30 ml) was added to the filtrate and it was extracted with three 30-ml portions of ethyl acetate. The residue obtained after evaporation of the ethyl acetate was dissolved in acetonitrile. TLC showed one major and three minor products. The major product was purified by HPLC (acetonitrile–water, 50:50). MS (70 eV) *m/z* 226 (M^+).

*[$^2H_9$]-2-Nitrofluorene (**15**).* This compound was prepared from [$^2H_{10}$] fluorene as described for **13**. MS (70 eV), *m/z* 220 (M^+).

*[$^2H_9$]-2-Aminofluorene (**16**).* This compound was prepared from [$^2H_9$]-1-nitrofluorene as described for **14**. MS (70 eV), *m/z* 190 (M^+).

*[$^2H_9$]-N-Heptafluorobutyryl-1-aminopyrene (**1a**) and [$^2H_9$]-N-heptafluorobutyryl-2-aminofluorene (**9a**).* These compounds were prepared as described for **1** and **9** starting with [$^2H_9$]-1-AP and [$^2H_9$]-2-AF, respectively. Product **1a**: MS (70 eV), *m/z* 422 (M^+). Product **9a**: MS (70 eV), *m/z* 386 (M^+).

*[$^2H_9$]-N-Heptafluorobutyryl-N-methyl-1-aminopyrene (**2a**) and [$^2H_9$]-N-heptafluorobutyryl-N-methyl-2-aminofluorene (**10a**).* These compounds were prepared from **1a** and **9a**, respectively, using the synthetic procedures for compounds **2** and **10**. Product **2a**: MS (70 eV), *m/z* 436 (M^+). Product **10a**: MS (70 eV), *m/z* 400 (M^+).

*N-Heptafluorobutyryl-N-([$^2H_3$]methyl)-1-aminopyrene (**2b**) and N-heptafluo-*

*robutyryl-N-([$^2H_3$]methyl)-2-aminofluorene (**10b**)*. These compounds were prepared from compounds **1** and **9** using [$^2H_3$]methyl iodide and the synthetic procedures described for **2** and **10**. Product **2b**: MS (70 eV), *m/z* 430 (M^+). Product **10b**: MS (70 eV), *m/z* 394 (M^+). Consistent with the isotopic purities provided by the supplier of the deuterium-labelled precursors, no evidence of [$^2H_8$] ring or [$^2H_2$]methyl products was seen in the EI mass spectra of the above deuteriated compounds.

Hydrolytic stability of N-pentafluorobenzylidenyl derivatives of 1-AP and 2-AF

A pH 6 stock solution of 100 m*M* buffer was prepared with KH_2PO_4 and K_2HPO_4. Solutions varying in pH from 3 to 12 were made by adjusting the stock solution with H_3PO_4 or KOH–water (45:55, w/v). A volume of 495 μl of the solution of Schiff base containing 1 mg of the compound in 49.5 ml of acetonitrile was added to 1-ml Reactivials each containing 5 μl of the buffer solution of pH 3, 6, 7, 7.4, 8, 10 and 12, respectively. A 20-μl volume of this solution contained 400 ng of Schiff base. The vials were capped and vortexed. Each of the solutions was analyzed by HPLC at different time intervals.

RESULTS AND DISCUSSION

The goal of this work was to establish electrophoric derivatives of amino-PAHs which are most suitable for sensitive determination by GC–ECNI-MS. 1-AP was studied as a model compound and derivatives **1**–**8** (see Table I) were synthesized. Based on the ease of preparation and yield, compounds **1**–**3** and **8** were selected for further study. The corresponding derivatives of 2-AF were then prepared (compounds **9**–**12**, Table I).

All derivatives turned out to be attractive for sensitive detection by GC–ECNI-MS. From a synthetic standpoint, however, some of them were disappointing. Compound **6**, for example, was obtained in only a 15% yield, as was the side-product in this reaction, the *bis*-pentafluorobenzyl derivative **7** (20% yield). The yield of compound **4** was only 40%.

Potentially the analytical usefulness at trace levels of the Schiff base derivatives **8** and **12** would be compromised if they were suspectible to hydrolysis. It was encouraging to observe that these derivatives are stable at pH values above 6 for at least fifteen days.

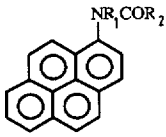
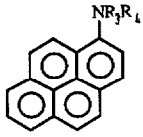
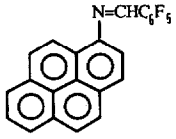
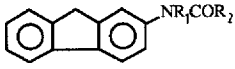
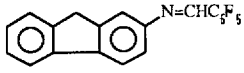
Electron-capture negative-ion mass spectra

ECNI mass spectra of all of the derivatives (with sample introduction by GC) are listed in Table II. As seen, each derivative yields one or more structurally diagnostic ions. Only the two pentafluorobenzylidene (Schiff base) derivatives (**8** and **12**) give M^- as a base peak. No molecular ion is observed at all for the other derivatives except the N-methyl-N-heptafluorobutyryl derivative **2** of 1-AP, which gives a moderately intense M^- peak relative to a base peak for $COC_3F_7^-$. The tendency of the other derivatives, depending on their structural features, to lose a hydrogen atom (HF) or a pentafluorobenzyl group (M − 181) accounts for their failure to exhibit a significant M^- ion.

GC–ECNI-MS using SIM of the most intense, characteristic ion of each derivative (data not shown) shows that each of the four AF derivatives exhibit approximately equal electron-capture response with the most intense one (Schiff base deriv-

TABLE I

ELECTROPHORIC DERIVATIVES OF 1-AMINOPYRENE AND 2-AMINOFLUORENE

Structure	*Number*	R_1	R_2
	**1**	H	C_3F_7
	2	CH_3	C_3F_7
	3	$CH_2C_6F_5$	C_3F_7
	4	H	C_6F_5
	5	CH_3	C_6F_5
		R_3	R_4
	**6**	H	$CH_2C_6F_5$
	7	$CH_2C_6F_5$	$CH_2C_6F_5$
	**8**	–	–
		R_1	R_2
	**9**	H	C_3F_7
	10	CH_3	C_3F_7
	11	$CH_2C_6F_5$	C_3F_7
	**12**	–	–

ative **12**) being only 40% stronger than the weakest. The GC elution order for the four compounds is **10**, **9**, **11**, **12**.

Comparison of the Schiff base derivative **12** with 2-NF shows that it elutes later, tails less and gives a 22-fold stronger response (molecular anion detection) under the ECNI conditions employed (Fig. 1). This response is linear over the range evaluated (0.30–250 pg). The detection limit under the same conditions for 2-NF is about 4 pg (signal-to-noise ratio = 5). The GC-electron-capture detection (ECD) molar response of the Schiff base derivative **12**, relative to that of lindane and aldrin, is 0.46 and 0.39, and that of 2-NF is 0.30 and 0.25, respectively. Thus, in contrast to their different responses by GC–ECNI-MS, **12** and 2-NF are similarly sensitive by GC–ECD.

TABLE II

ELECTRON-CAPTURE NEGATIVE-ION MASS SPECTRA OF 1-AMINOPYRENE AND 2-AMINOFLUORENE DERIVATIVES

Format: relative intensity (assignment). GC was used introduce the compounds into the mass spectrometer.

Compound	M^-	$M-HF$	$M-2HF$	$M-181$	*Other*
1	–	100	5	–	7 (COC_3F_7), 12 (M – H)
2	45	10	2	–	100 (COC_3F_7)
3	–	–	–	100	
4	–	70	5	–	50 (M – H), 100 (M – H–HF)
5	–	100	80	–	
6	–	100	–	–	
7	–	–	–	100	90 (M – 181 – HF), 60 (C_6F_5)
8	100	–	–	–	
9	–	100	5	–	
10	–	20	100	–	20 (COC_3F_7) 10 (M – 3HF)
11	–	–	–	100	
12	100	–	–	–	

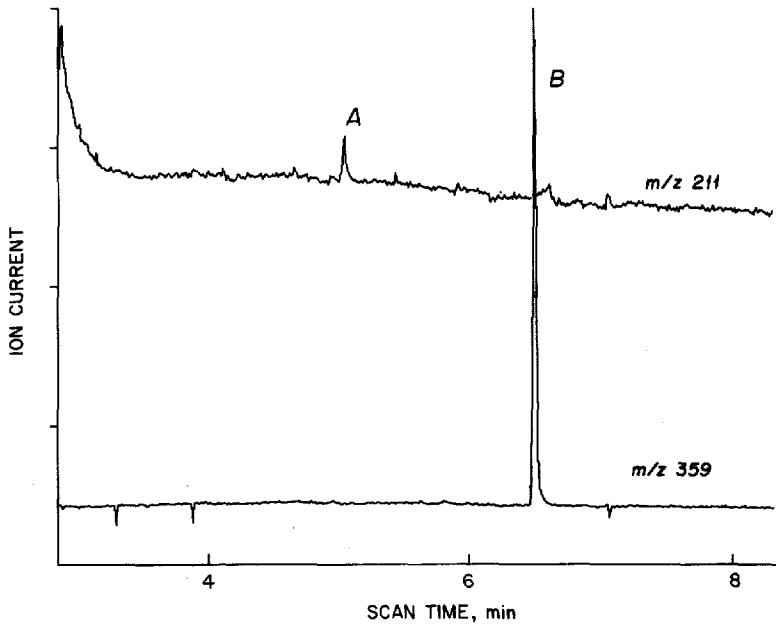
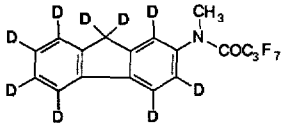


Fig. 1. GC–ECNI-MS profiles of 5.5 pg each of 2-nitrofluorene (A) and compound **12** (B). The mass chromatograms were recorded by monitoring the molecular anions *m/z* 211 (A) and *m/z* 359 (B).

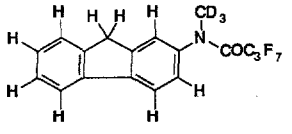
The ECNI spectra (data not shown) of compound **12** and 2-NF are both relatively simple. Aside from the molecular anion base peak (100% relative abundance) in each spectrum, that of **12** shows only one other ion ($M-HF^-$) with a relative abundance of 10–15%, and 2-NF similarly shows only a peak for $[M-O]^-$ at 4–5% relative abundance.

The molar response of **8** by GC–ECD, relative to lindane and aldrin, is 0.25 and 0.21, and that of 1-NP is similarly 0.18 and 0.14.

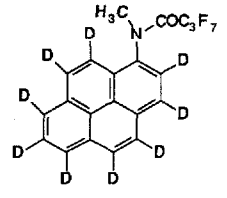
Several interesting differences were noted in the ECNI mass spectra of the fluorene and pyrene derivatives. Chief among these is the production of molecular ions and $[M-H]^-$ ions of moderate intensity (12–45%) for the heptafluorobutyryl pyrene derivatives **1** and **2** and their absence in the corresponding fluorene analogues **9** and **10** (Table II). To explore these differences, we prepared certain deuterated analogues, the structures of some of which are shown below.



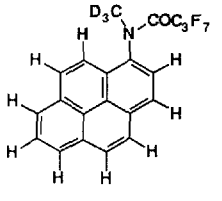
10 a



10 b



2 a



2 b

Examination of the spectra for compounds **1a** and **9a** (data not shown) reveals that the elimination of the amino hydrogen, when present, is predominant in the initial loss of HF for these compounds, not an unexpected finding[a] (ref. 18).

Comparison of the spectra of the $[^2H_9]$ ring and $[^2H_3]$methyl derivatives of compounds **2a**, **2b**, **10a** and **10b** (Fig. 2, Table III) also shows a preference for the site of the hydrogen removal in the loss of HF. The true preference may be somewhat obscured by the potential for $H/^2H$ scrambling and isotope effects. Nevertheless, the fluorene derivative **10**, which exhibits no molecular anion, clearly favors loss of the

[a] Under conditions of increasing sample pressure, however, the situation changed. Injections of 100 ng or more produced ECNI mass spectra where not only was the ratio of the loss of $HF/^2HF$ near 1:1, but the dominant fragment 3:1 became $[M-F]^-$. These observations underscore the dependence of ECNI mass spectra on sample amounts including the associated changes in reactive species in the ion source.

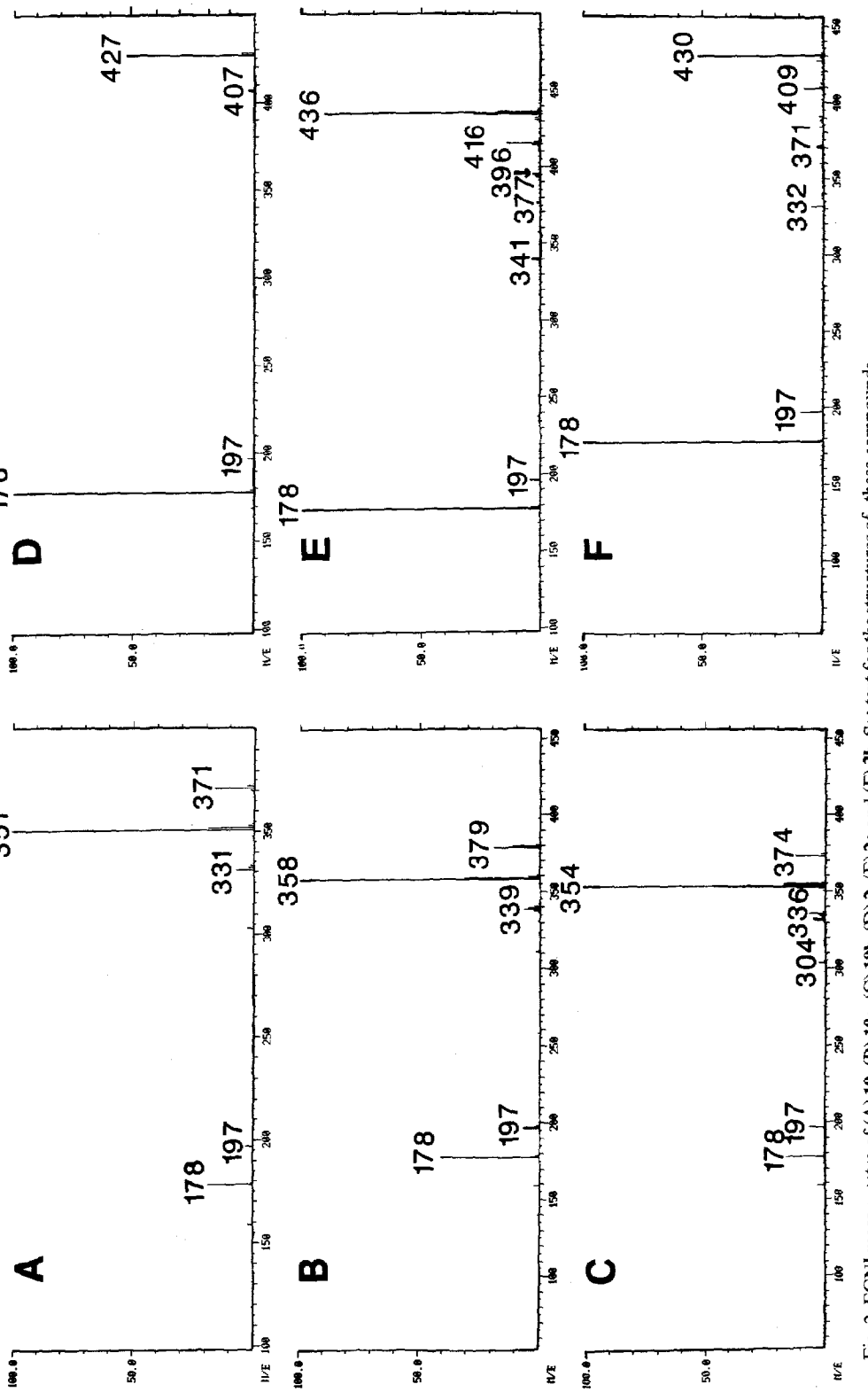


Fig. 2. ECNI mass spectra of (A) **10**, (B) **10a**, (C) **10b**, (D) **2**, (E) **2a** and (F) **2b**. See text for the structures of these compounds.

TABLE III

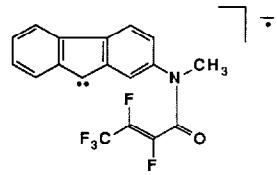
ORIGIN OF H (AS H OR ^{2}H) IN THE LOSS OF HF AND TWO HF

The values in parentheses are the relative abundances. These are corrected for isotopic contributions from the *m/z* at 1 lower as necessary.

Fragment	*m/z*			
	Fluorene derivative		*Pyrene derivative*	
	10a *(ring-d_9)*	***10b*** *([$^2H_3$]methyl)*	***2a*** *(ring-d_9)*	***2b*** *([$^2H_3$]methyl)*
M − HF	378 (7.5)	374 (12.5)	416 (6.2)	410 (−)
M − ^{2}HF	379 (−)	375 (−)[a]	415 (1.8)	409 (8.2)
M − 2HF	360 (−)	354 (98.0)	396 (1.2)	390 (0.5)
M − 2^2HF	358 (100)	352 (−)	394 (0.8)	388 (3.0)
M − (HF + ^{2}HF)	359 (11.2)	353 (11.3)	395 (0.8)	389 (1.0)

[a] No peak was evident.

ring hydrogens in both the first and especially the second HF elimination. The removal of two molecules of HF can increase the degree of conjugation in both the ring system and the side-chain, assuming that an ion of the following structure is formed (*m/z* 351). Thus it is not surprising that this ion is the base peak in the spectrum of **10**.



m / z 351

In the pyrene derivative **2**, there is little tendency to lose either one HF (6–8% relative abundance) or two HF (1% relative abundance). The *N*-methyl group provides more reactive hydrogens for this purpose than does the pyrene ring system.

The absolute intensity of the base peak in the ECNI mass spectra of compounds **9–12** as a function of changing ion source temperature is shown in Fig. 3. For reasons that are unclear, temperatures above 230°C increase the intensity of these ions. The other ions in the spectra of these derivatives exhibit a parallel decline in intensity (data not shown). In contrast, other[21,22] have reported that an increase in source temperature causes increased fragmentation of compounds. Potentially a hotter source can stay cleaner and provide increased sensitivity.

Positive-ion chemical-ionization mass spectra (data not shown)

The positive-ion chemical-ionization mass spectra of the derivatives show an $[M+H]^+$ ion as the base peak for all of the compounds except **3**, **7** and **11**, in which a pentafluorobenzyl (PFB) group is substituted on a tertiary nitrogen. In this case

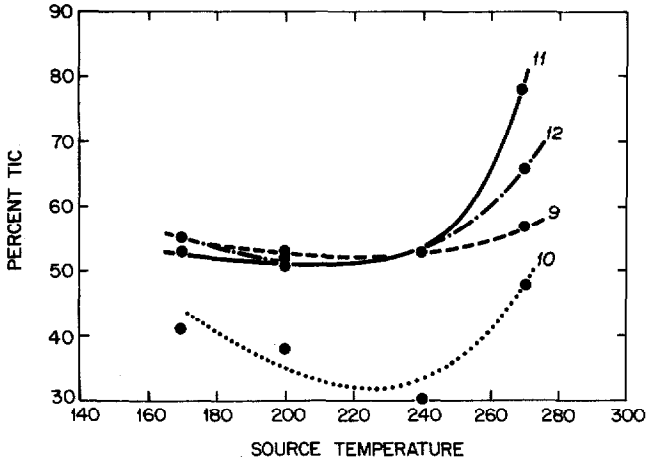


Fig. 3. Intensity of base peak (*m/z* 376 for **11**, *m/z* 359 for **12**, *m/z* 352 for **9** and *m/z* 351 for **10**) relative to total ion current (TIC) as a function of source temperature.

$[M-PFB]^+$ and $[PFB]^+$ ions are the most intense peaks. $[M-HF]^+$ ions are common in many of the spectra.

Electron-impact mass spectra (data not shown)

The EI mass spectra of the N-acyl derivatives contain an M^+ ion as the base peak and exhibit limited fragmentation. Loss of C_3F_7 or C_6F_5 with the subsequent loss of H, loss of the acyl group, and formation of a cyclopentadienyl ring through loss of HCN are common to all of the derivatives.

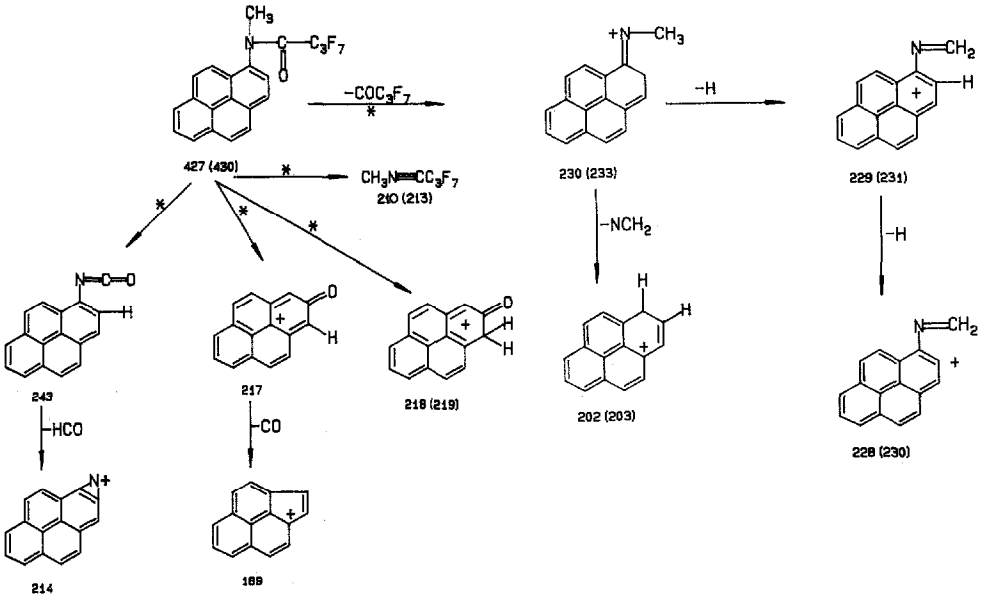
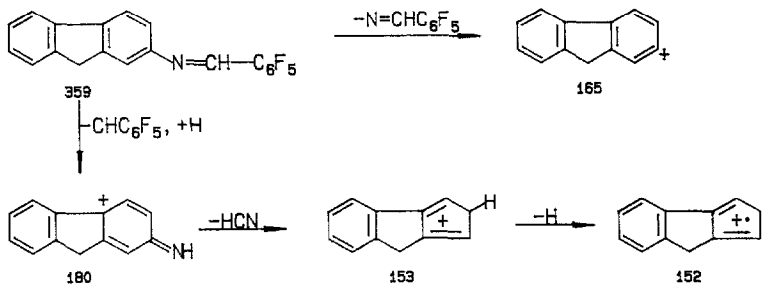


Fig. 4. Fragmentation scheme for compound **2** under EI conditions.

All of the N-alkyl-N-acyl derivatives yield M^+ ions, although in the N-PFB compounds the abundance of this ion is reduced to 20–30%. Fragmentations typical of these compounds are shown in Fig. 4, using N-methyl-N-heptafluorobutyryl-1-aminopyrene as a representative example. Ions of moderate intensity are formed from the loss of the heptafluorobutyryl or PFB group and from the loss of both groups substituted on the nitrogen. The base peak in the N-PFB derivatives **3** and **12** results from the formation of an isocyanate ion. This ion is also formed in the N-methyl derivatives, although only in 5–15% relative abundance. This difference can no doubt be attributed to the facile cleavage of the PFB group as opposed to the methyl group. This is further supported by the absence of $[M-15]^+$ ions in the spectra of **2** and **10**.

An interesting rearrangement occurs to a varying extent in the N-alkyl-N-acyl derivatives **2**, **5** and **10**. This involves cleavage of the C–N bond with a transfer of the carbonyl oxygen to the ring, the charge being shared on both fragments. High-resolution accurate mass measurements on **2** indicate that when the charge remains on the oxygen, an ion is formed at *m/z* 217 with an elemental composition of $C_{16}H_9O$. Charge retention on the nitrogen forms a very stable ion at *m/z* 210 ($C_5H_3NF_7$) in the spectrum of **2** and **10**. This ion is shifted in the spectrum of **5** to *m/z* 207. No corresponding ion at 217 or 218, however, is found in the spectrum of **5**, presumably due to the prevention of charge migration by the pentafluorobenzamide group (See Fig. 4).

The benzylidine derivatives **8** and **12** largely fragment as follows:



CONCLUSION

Of the electrophoric derivatives that we have prepared of 1-AP and 2-AF, the best one overall is a pentafluorobenzylidene derivative. It forms rapidly in high yield, is hydrolytically stable above pH 6 and gives an intense molecular anion by GC–ECNI-MS. It yields structurally diagnostic ions by positive-ion chemical-ionization MS and EI-MS. Potentially this derivative will continue to be successful when applied to the determination of trace amounts of these and related aminopolyaromatic adducts from biological samples. The N-pentafluorobenzyl-N-heptafluorobutyryl derivative also is attractive.

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