[CONTRIBUTION FROM THE CANCER RESEARCH LABORATORY, UNIVERSITY OF FLORIDA]

Nitration of 2-Acylaminofluorene Derivatives[1]

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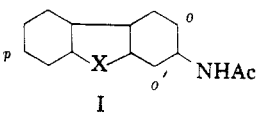
The nitration of 2-acetylaminofluorene, 2-AAF, has been found to take place approximately 60% in the 3-position, 25% in the 7-position, and <1% in the 1-position. Blocking the 7-position of 2-AAF with a methyl or ethyl group increased the yield of 3-nitro compound to 80–90%. Replacement of the acetyl group in 2-AAF by the methanesulfonyl group increased the yield of 3-nitro derivative to about 80%. On the other hand nitration of 2-*n*-perfluorobutyrylaminofluorene occurred in approximately 80% yield in the 7-position. Thus the position of substitution in these cases is dependent on the electronegativity of the N-acyl group. Thirteen new compounds have been prepared. The absorption spectra of 14 compounds have been discussed.

The nitration of 2-acetylaminofluorene, 2-AAF, has been studied by several groups.[2-5] From an examination of this literature it can be crudely estimated that the nitration takes place 60% in the 3-position and 25% in the 7-position.[6] The nitration of 2,7-diacetylaminofluorene gave an 84% yield of the pure 3,6-dinitro derivative.[7] Similarly the chlorination[8] and the mercuration[9] of 2-aminofluorene, 2-AF, occurred mainly, if not exclusively, in the 3-position. On the other hand 2-AAF and 2-*p*-toluenesulfonylaminofluorene are brominated mainly in the 7-position with some attack at the 3-position.[6] 2-AF forms with mesoxalic ester an indenoisatin which is ring-closed in the 3-position.[10] However, 2-AF and ethyl ethoxymethylenemalonate gave a product which was proven to be ring-closed in the 1-position.[11] The nitration of 2-*p*-toluenesulfonylaminofluorene,[6] 2-carbethoxyaminofluorenone,[3] and 2-benzoylaminofluorenone[3] gave mainly, if not exclusively, the 3-nitro derivative.

An examination of the nitration of analogous acetylamino derivatives of fluorene, I, X = CH_2; dibenzofuran, I, X = O; dibenzothiophene, I, X = S; dibenzoselenophene, I, X = Se; and 9-methylcarbazole, I, X = NMe, has shown that while I, X = CH_2, was nitrated mainly in the *o*- and extended *p*-positions; I, X = O, was nitrated 75% in the *o*-position;[12] I, X = S, was nitrated 77% in the *o'*-position;[13] I, X = Se, was nitrated 70% in the *o'*-position;[14] and I, X = NMe, was nitrated 40% in the *o*-position.[15]



I

This bewildering variation in the nitration of the I analogs should be capable of correlation with the electronic distributions in these molecules.

Substitutions in 2-acylaminofluorenes or 2-AF would appear to occur mainly in the 1-, 3-, or 7-positions with the 3-position favored. The course of these substitutions is apparently dependent on such factors as the entering group, the directing group and the solvent. In this paper the effect of a change in the acyl group on the nitration of 2-acylaminofluorenes is described. In this respect the effect of blocking the 7-position on the course of nitration was also of interest.

The nitration of 2-AAF was reinvestigated. The crude nitroamines, obtained from the crude nitro AAF mixture, were chromatographed in xylene solution using benzene as a developer. The first product through the column was found to melt at 161–163° after purification. The second product on the column was 3-nitro-2-AF, m.p. 202–203°, and the third product, was 7-nitro-2-AF, m.p. 231–232°. The new compound, m.p. 161–163°, is spectrally similar to 3-nitro-2-AF and contains an aromatic *o*-nitroamino band at max 465 mμ, log ε 3.70. Other types of nitroaminofluorene derivatives such as 5-nitro-2-AF[16] and 2-nitro-5-AF[17] absorb at considerably shorter wave length and do not show the

(1) This investigation was supported by research grant C-1066 from the National Cancer Institute, U. S. Public Health Service.

(2) Diels, Schell, and Tolson, *Ber.*, **35**, 3286 (1902).

(3) Eckert and Langecker, *J. prakt. Chem.*, **118**, 263 (1928).

(4) Porai-Koshits and Nikiforova, *J. Appl. Chem. U.S.S.R.*, **13**, 215 (1940); *Chem. Abstr.*, **35**, 625 (1941).

(5) Bardout, *Anales asoc. quim. argentina*, **19**, 117 (1931); *Chem. Abstr.*, **26**, 1275 (1932).

(6) Bell and Mulholland, *J. Chem. Soc.*, 2020 (1949).

(7) Barker and Barker, *J. Chem. Soc.*, 870 (1954).

(8) Bell and Gibson, *J. Chem. Soc.*, 3560 (1955).

(9) Weisburger, Weisburger, and Ray, *J. Org. Chem.*, **16**, 1697 (1951).

(10) Campbell and Stafford, *J. Chem. Soc.*, 299 (1952).

(11) Bremer and Hamilton, *J. Am. Chem. Soc.*, **73**, 1844 (1951).

(12) Gilman and Wilder, *J. Am. Chem. Soc.*, **76**, 2906 (1954).

(13) Sawicki, *J. Org. Chem.*, **18**, 1492 (1953).

(14) Sawicki, *J. Org. Chem.*, **19**, 1163 (1954).

(15) Sawicki, *J. Am. Chem. Soc.*, **76**, 664 (1954).

(16) Weisburger and Weisburger, *J. Org. Chem.*, **21**, 514 (1956).

(17) Weisburger and Weisburger, *J. Org. Chem.*, **20**, 1396 (1955).

fine structure near 300 mμ characteristic of the 1-nitro and 3-nitro derivatives, Table I. The spectrum of the new nitroamine in alcoholic sulfuric acid is radically different from the spectrum of 2-nitrofluorene and definitely different from the spectrum of 4-nitrofluorene,[18] but similar to the spectrum of 3-nitro-2-AF in alcoholic sulfuric acid, Table I. Consequently the compound is 1-nitro-2-AF.

TABLE I

SPECTRAL DATA OF NITROFLUORENE DERIVATIVES IN 95% ETHANOL

Compound	$\lambda_{max.}$ in mμ (log ε)			
1-Nitro-2-AF[a]	281 (4.52)	*300*[b] (4.35) *306* (4.32)	464–468	(3.70)
1-Nitro-2-AF[c]	262 (4.41)	*285* (4.19) 294 (4.09)	*336*	(3.11)
3-Nitro-2-AF	274 (4.50)	*296* (4.3) *304* (4.24)	442–449	(3.73)
3-Nitro-2-AF[c]	259 (4.42)	*285* (4.19) 294 (4.08)	334	(3.14)
3-Nitro-7-methyl-2-AF	275 (4.51)	*300* (4.27) *306* (4.2)	450–452	(3.71)
3-Nitro-7-methyl-2-AF[c]	264 (4.44)	287 (4.24) 298 (4.19)	332	(3.19)
3-Nitro-7-ethyl-2-AF	276 (4.52)	*300* (4.30) *306* (4.25)	450–452	(3.71)
3-Nitro-7-ethyl-2-AF[c]	264 (4.46)	287 (4.27) 298 (4.23)	336	(3.11)
3-Nitro-2-mesyl AF	264 (4.49) *272* (4.44)	287 (4.29) 296 (4.25)	382	(3.26)
3-Nitro-2-p-tosyl AF	266 (4.40) *274* (4.38)	289 (4.28) 298 (4.27)	382	(3.21)
7-Nitro-2-AF	262 (4.09)	400 (4.27)		
7-Nitro-2-p-tosyl AF	245 (4.10)	347 (4.31)		
5-Nitro-2-AF[16]	280 (4.17)	406 (3.73)		
2-Nitro-5-AF[17]	275 (3.87) 329 (4.02)	377 (4.03)		
2-Nitrofluorene	233 (3.98)	331 (4.26)		
4-Nitrofluorene[18]	255 (4.33)	335 (3.80)		

[a] AF is aminofluorene. [b] *Underlined* values are shoulders. [c] In alcoholic 25% sulfuric acid.

The nitration of either 7-methyl- or 7-ethyl-2-AAF gave an 80–90% yield of mononitro compound. Deacetylation, reduction to the diamine, followed by reaction with selenium dioxide formed piaselenoles which gave a positive test for selenium and a brilliant blue-green color in sulfuric acid. This proved that nitration occurred *ortho* to the acetylamino group. The derived nitroamines of the 7-alkyl compounds were closely similar spectrally to 3-nitro-2-AF, Table I. The slight bathochromic shift is evidently caused by the 7-alkyl group. On the other hand 1-nitro-2-AF, although spectrally similar to the 7-alkyl compounds, has its long wave length maxima shifted to the red 15–20 mμ as compared to 3-nitro-2-AF and the 7-alkyl nitroamines. It would appear that the nitration of 7-methyl or 7-ethyl-2-AAF occurred in the 3-position. Examination of the infrared spectra in a potassium bromide wafer of 3-nitro-2-AF and its 7-alkyl derivatives disclosed that these three compounds have a closely similar spectral pattern in the 6–10μ region with the most intense band occurring at 8.0μ. On the other hand, 1-nitro-2-AF has an entirely different spectral pattern, especially in the 7–10μ region, with the strongest band occurring at 6.08μ.

The nitration of 2-*n*-perfluorobutyrylaminofluorene gave an 80% yield of a mononitro compound, m.p. 205–206°. Alkaline hydrolysis gave 7-nitro-2-AF, m.p. 231–232°. Reaction of authentic 7-nitro-2-AF[19] with perfluorobutyric anhydride gave the acyl derivative, m.p. 206–207°. The mixture melting point was 206–207°.

The nitration of 2-methylsulfonylaminofluorene gave a mononitro compound, m.p. 211–212°, in 80% yield. As 7-nitro-2-methylsulfonylaminofluorene melts at 239–240°, it is probable that the nitration took place in the 3-position. This is in line with the nitration of 2-*p*-toluenesulfonylaminofluorene which occurred in the 3-position.[6] As the spectra of nitro-2-methylsulfonylaminofluorene and 3-nitro-2-*p*-toluenesulfonylaminofluorene are similar, Table I, it would appear that nitration of 2-methylsulfonylaminofluorene took place in the 1- or 3-position. Further, as nitration of 2-acylaminofluorenes has been shown to occur in the 3- and/or 7-positions almost exclusively, the nitro group is tentatively assigned the 3-position.

Examination of the spectral data in Table I discloses the following relationships. The 1- and 3-nitrofluorene derivatives had three groups of bands—an intense band at 260–280 mμ, two inflection points of slightly lower intensity near 300 mμ, and a low intensity longer wave length band. The long wave length bands at approximately 335 mμ apparently stem mainly from the nitrobenzene portion of the molecule. In this respect *o*-nitrotoluene has been reported as having a prominent low intensity shoulder at about 340 mμ in its ultraviolet spectrum.[20] The long wave length bands at approximately 450 mμ are derived from the *o*-nitroaniline portion of the molecule as modified by the extra-conjugative effect of the portion of the molecule *para* to the amino group. The intense band at 260–280 mμ with the 2 shoulders near 300 mμ is mainly derived from the fluorene portion of the molecule. This type of spectral envelope has been found for many types of fluorene derivatives.[21] On the other hand in the 2- and 4-nitrofluorenes the nitro group is in conjugation with both benzene rings and so the fluorene type of spectral envelope is lost.

An interesting point that needs to be considered is the carcinogenicity of 2-acylaminofluorenes as related to such factors as the electronegativity of the acyl group and the consequent directive influence

(18) Weisburger and Weisburger, *J. Org. Chem.*, **19**, 964 (1954).

(19) Cislak and Hamilton, *J. Am. Chem. Soc.*, **53**, 746 (1931).

(20) Dede and Rosenberg, *Ber.*, **67**, 147 (1934).

(21) Sawicki, *J. Org. Chem.*, **21**, 271 (1956).

of the acylamino group. 2-*p*-Toluenesulfonylaminofluorene is non-carcinogenic[22] and is nitrated in the 3-position; 2-AAF is a strong carcinogen[23] and is nitrated in the 3- and 7-positions; 2-trifluoroacetylaminofluorene is the strongest carcinogen of this series in the rat[23] and the related derivative 2-*n*-perfluorobutyrylaminofluorene is nitrated in the 7-position. Admittedly other factors such as the ease of deacylation, the solubility, and intrinsic factors of various kinds would have an effect on the cancer-producing activity of these chemicals, but it is apparent that the effect of various acyl groups on the chemical carcinogenic process needs to be more thoroughly studied.

EXPERIMENTAL[24]

1-Nitro-2-AF. Nitration of 2-AAF by the procedure of Diels, *et al.*[3] gave a crude nitrated mixture which was hydrolyzed with boiling Methyl Cellosolve-hydrochloric acid. The nitroamine mixture dissolved in xylene was chromatographed with benzene as a developer. On the alumina column in ascending order was found a well-separated pink layer of 1-nitro-2-AF, a broad red layer of 3-nitro-2-AF, a red layer of 7-nitro-2-AF, and at the surface a dirty brown layer. The pink layer was pulled through the column while the other layers were cut and eluted with acetone or acetic acid. The compound, m.p. 136–152°, obtained from the pink layer was crystallized twice from hexane to give <1% yield of red needles, m.p. 161–163°.

Anal. Calc'd for $C_{13}H_{10}N_2O_2$: C, 69.0; H, 4.42. Found: C, 68.9; H, 4.22.

3-Nitro-7-methyl-2-AAF. To a stirred solution of 4.8 g. (0.02 mole) of 7-methyl-2-AAF in 55 ml. of acetic acid at 55–60° was added dropwise 2.4 ml. of nitric acid (*d.* 1.40). Stirring was maintained an additional 20–30 minutes after the mixture solidified. Crystallization from Methyl Cellosolve or xylene gave 4.5 g. (80%) of yellow cottony needles, m.p. 252°. The compound can also be crystallized from nitrobenzene or acetic acid.

Anal. Calc'd for $C_{16}H_{14}N_4O_3$: C, 68.1; H, 4.96; N, 9.93. Found: C, 67.8; H, 5.03; N, 9.56.

3-Nitro-7-ethyl-2-AAF. Using the procedure for the 7-methyl analog, 10 g. of 7-ethyl-2-AAF gave, after crystallization from xylene or a large volume of alcohol, 10.0–10.5 g. (85–89%) of yellow needles, m.p. 225–226°.

Anal. Calc'd for $C_{17}H_{16}N_2O_3$: C, 68.9; H, 5.41. Found: C, 69.1; H, 5.35.

3-Nitro-7-methyl-2-AF. A suspension of 2.82 g. (0.001 mole) of 7-methyl-3-nitro-2-AAF in 50 ml. of Methyl Cellosolve and 20 ml. of concentrated hydrochloric acid was vigorously refluxed for 2–3 hours. The clear red solution was poured into excess water. Crystallization from xylene gave 2.94 g. (90%) of red needles, m.p. 199–200°.

Anal. Calc'd for $C_{14}H_{12}N_2O_2$: N, 11.7. Found: N, 11.8.

3-Nitro-7-ethyl-2-AF. Hydrolysis of the AAF derivative followed by crystallization from methanol gave an 85–95% yield of red needles, m.p. 160–161°.

Anal. Calc'd for $C_{15}H_{14}N_2O_2$: C, 70.9; H, 5.51; N, 11.0. Found: C, 71.1; H, 5.4; N, 11.0.

3-Amino-7-methyl-2-AF. A solution of 0.7 g. of calcium chloride in 14 ml. of water followed by 21 g. of zinc dust was added to a boiling solution of 2.4 g. (0.01 mole) of 3-nitro-7-methyl-2-AF in 60 ml. of 95% ethanol. The mixture was vigorously refluxed for 4 hours and then was filtered.

The zinc was extracted twice with 10-ml. portions of boiling Methyl Cellosolve. The filtrate and the extracts were poured into excess water. Crystallization from heptane-benzene gave 1.8 g. (85% yield) of colorless crystals m.p. 183–184°.

Anal. Calc'd for $C_{14}H_{14}N_2$: N, 13.3; Found: N, 13.2.

3-Amino-7-ethyl-2-AF. The same reduction procedure was used. Crystallization from benzene gave a 70–80% yield of colorless crystals m.p. 198–199°.

Anal. Calc'd for $C_{15}H_{16}N_2$: N, 12.5. Found: N, 12.3.

7-Methylindeno[1,2-f]piaselenole. A solution of 0.21 g. (0.001 mole) of 3-amino-7-methyl-2-AF and 0.12 g. of selenium dioxide in 10 ml. of alcohol was refluxed for 15–30 minutes. Ten ml. of water was added. Crystallization from heptane or methanol gave 0.25 g. (87%) of yellow plates, m.p. 159–160°. The compound dissolved in sulfuric acid with a dark blue-green color.

Anal. Calc'd for $C_{14}H_{10}N_2Se$: C, 59.0; H, 3.51; N, 9.82. Found: C, 59.1; H, 3.74; N, 9.49.

7-Ethylindeno[1,2-f]piaselenole. Prepared in a similar fashion from the diamine and selenium dioxide this compound was obtained as light yellow plates or needles, m.p. 131–132°, in 80–90% yield after crystallization from hexane. Solution of the compound in sulfuric acid gave a dark blue-green color.

Anal. Calc'd for $C_{15}H_{12}N_2Se$: C, 60.2; H, 4.01; N, 9.4. Found: C, 60.5; H, 4.3; N, 9.4.

7-Nitro-2-n-perfluorobutyrylaminofluorene. (*a*). Two ml. of nitric acid (*d.* 1.5) was added at 64° to a suspension of 3.77 g. (0.01 mole) of 2-*n*-perfluorobutyrylaminofluorene in 25 ml. of acetic acid. The temperature shot up to 75° and the mixture solidified. Crystallization from benzene and then methanol gave 3.38 g. (80%) of yellow needles, m.p. 205–206°.

Anal. Calc'd for $C_{17}H_9F_7N_2O_3$: C, 48.3; H, 2.13; N, 6.63. Found: C, 48.6; H, 2.30; N, 6.33.

Hydrolysis of this product with alcoholic potassium hydroxide gave 7-nitro-2-AF, m.p. 229–231°. Mixture melting point with authentic compound was 230–232°.

(*b*). A solution of 7-nitro-2-AF in hot xylene was treated with an equivalent of *n*-perfluorobutyric anhydride. Isolation by the usual procedure and crystallization from benzene gave a 95% yield of yellow needles, m.p. 206–207°. Mixture melting point with compound in (*a*) was 206–207°.

3-Nitro-2-n-perfluorobutyrylaminofluorene. Solution of 3-nitro-2-AF in hot excess *n*-perfluorobutyric anhydride containing a drop of sulfuric acid followed by the addition of excess water in 15 minutes gave a yellow precipitate. Crystallization from heptane gave a 95% yield of yellow needles, m.p. 156–157°.

Anal. Calc'd for $C_{17}H_9F_7N_2O_3$: C, 48.3; H, 2.13. Found: C, 48.1; H, 2.35.

3-Nitro-2-methylanesulfonylaminofluorene. The addition of 2.7 ml. of nitric acid (*d.* 1.5) in 7 ml. of acetic acid to a suspension of 2.6 g. (0.01 mole) of 2-methanesulfonylaminofluorene in 30 ml. of acetic acid at 40° resulted in a clear solution which soon solidified. Excess water was added. Crystallization from alcohol and then benzene gave 2.4 g. (80%) of yellow needles, m.p. 211–212°. Attempts to hydrolyze the compound with alcoholic hydrochloric acid or concentrated sulfuric have been unsuccessful.

Anal. Calc'd for $C_{14}H_{12}N_2O_4S$: N, 9.21. Found: N, 9.27.

7-Nitro-2-methanesulfonylaminofluorene. One ml. of methanesulfonyl chloride was added dropwise to an ice-cold solution of 2.26 g. (0.01 mole) of 7-nitro-2-aminofluorene in 50 ml. of pyridine. Thirty minutes after the addition excess water was added. Crystallization from acetic acid gave 2.74 g. (90%) of yellow crystals, m.p. 239–240° dec.

Anal. Calc'd for $C_{14}H_{12}N_2O_4S$: N, 9.21. Found: N, 9.16.

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(22) Ray and Argus, *Cancer Research*, 11, 783 (1951).

(23) Morris, *J. Natl. Cancer Inst.*, 15, 1535 (1955).

(24) All melting points are uncorrected. Analyses are by Peninsular ChemResearch, Inc., Gainesville, Florida.