ROUTE TO THE TRITIATION OF CARBON ATOM-9 OF CARCINOGENIC FLUORENYLHYDROXAMIC ACIDS.

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SUMMARY

The selective tritiation of the methylene carbon atom of the carcinogens, N-fluoren-3-yl-and N-fluoren-1-ylacetohydroxamic acid, has been investigated. The synthesis of N-(9-^{3}H)fluoren-3-ylacetohhydroxamic acid involves hydrogenolysis of 3-aminofluoren-9-one to (9-^{3})fluoren-3-amine with $LiAl^3H_4$-$AlCl_3$. The tritiated amine is oxidized to 3-nitro-(9-^{3}H)fluorene with m-chloroperoxybenzoic and the labeled nitro compound is partially hydrogenated to the ^{3}H-hydroxamic acid with 10% Pd-C catalyst in presence of acetic anhydride and triethylamine or dimethylaniline. N-(9-^{3}H)fluoren-1-ylacetohydroxamic acid may be obtained from 1-aminofluoren-9-one by the same procedure.However, N-(9-^{3}H)fluoren-2-ylacetohydroxamic acid cannot be prepared in this way because reduction of 2-aminofluoren-9-one with $LiAlH_4$-$AlCl_3$ does not proceed beyond the alcohol, 2-aminofluoren-9-ol.

INTRODUCTION

N-Fluoren-3- and -1-ylacetohydroxamic acids have recently been shown to be carcinogens for the rat[1]. Neither the metabolism nor the interaction of these carcinogens with cell constituents has been investigated in depth, mainly because a method to label these carcinogens isotopically was not avail-

able. Since the method for the synthesis of N-[9-^{14}C]fluoren-2-ylacetohydroxamic acid[2,3] was not readily applicable to the isomeric fluorenylhydroxamic acids, labeling of these compounds with ^{3}H was felt to be the most promising approach. The general labeling of the fluorene moiety by tritium exchange appeared feasible. However, it seemed preferable to tritiate specifically carbon atom-9 because if the molecule were generally labeled the distribution of ^{3}H in the fluorene system would be unknown and could not be readily determined. Furthermore, there is no evidence, at least in the case of N-fluoren-2-ylacetamide or of N-fluoren-2-ylacetohydroxamic acid, that the hydrogen atoms at the methylene carbon atom, unlike those linked to the carbon atoms of the aromatic rings or to the nitrogen, are replaced metabolically.

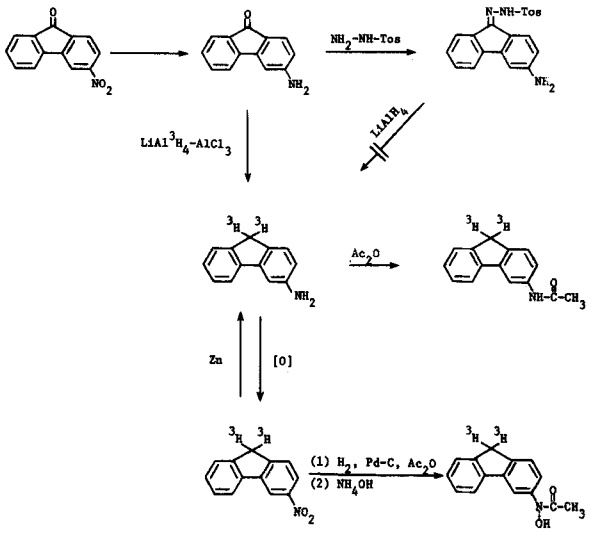
The synthesis leading to N-[9-^{3}H]fluoren-3- or -1-ylacetohydroxamic acid is outlined in Figure 1. The step in which the ^{3}H is introduced into the desired position is the hydrogenolysis, with $LiAl^{3}H_4$-$AlCl_3$, of the respective aminofluorenone to the amine. The fluorenamines could then be oxidized with m-chloroperoxybenzoic acid to the nitrofluorenes[4] and the labeled hydroxamic acids would be available from the catalytic reduction of the nitro compounds in presence of acetic anhydride[5]. The reduction of the nitrofluorenones to the nitrofluorenes which could conceivably be effected with $LiAlH_4$ was not feasible because of the insolubility of the nitroketones in the solvents ordinarily employed in this type of reduction, such as diethyl ether or THF.

Several options were explored to accomplish the reduction of aminofluorenones to the amines. The hydrogenolysis of the tosylhydrazones of steroidal ketones by a 17- to 18-fold molar excess of $LiAlH_4$[6] suggested that this procedure might be utilized for the introduction of ^{3}H at carbon atom-9 of the fluorene moiety. However, a 15- to 19-fold molar excess of $LiAlH_4$ failed to hydrogenolyze the tosylhydrazones of 3- and 1-aminofluoren-9-one. Under the same conditions the tosylhydrazone of 2-aminofluoren-9-one yielded only 7% of fluoren-2-amine.

The report that hydrogenolysis of certain substituted aromatic ketones,

Figure 1

Reaction Sequences for the Preparation of N-[9-^{3}H]fluoren-3-ylacetohydroxamic Acid and N-[9-^{3}H]fluoren-3-ylacetamide



aldehydes and carboxylic acids may be effected with excess $LiAlH_4$ and reaction times varying from 1 hr. to 11 days[7] prompted us to attempt the reduction of 1-aminofluoren-9-one with a 10-fold molar excess of $LiAlH_4$. After a reaction time of 72 hrs. the yield of fluoren-1-amine was only 7% and this method of reduction was abandoned.

Satisfactory yields of fluoren-3- and -1-amine were realized with the use of $LiAlH_4$-$AlCl_3$[8]. In the reduction of 3-aminofluoren-9-one yields ranging from 60-80% were obtained when the molar ratios of $LiAlH_4$-$AlCl_3$/aminoketone were 3.5-4.0 (Table 1). The somewhat lower yield of amine in the reduction of 1-aminofluoren-9-one (44%) is likely due to the fact that the reduction of the

TABLE 1

THE YIELD OF AMINOFLUORENES IN THE REDUCTION OF AMINOFLUORENONE BY $LiAlH_4$-$AlCl_3$

Aminofluorenone	mmoles	$LiAlH_4$ (mmoles)	$AlCl_3$ (mmoles)	Aminofluorene (mmoles)	%
3-aminofluoren-9-one	1.32	3.2[1]	4.5	0.42[2]	32
3-aminofluoren-9-one	1.29	4.8	4.5	0.76[2]	59
3-aminofluoren-9-one	1.32	4.8	4.6	0.82[2]	62
3-aminofluoren-9-one	1.30	4.8	4.5	0.93[2]	71
3-aminofluoren-9-one	1.30	4.8	4.5	1.03[2]	79
1-aminofluoren-9-one	1.54	5.4[3]	5.0	0.67[4]	44
2-aminofluoren-9-one	1.30	4.7	4.5	0.03[5]	2
2-aminofluoren-9-one	1.32	4.7	4.5	0.01[5,6]	<1

[1] In this run 1.6 mmoles of $LiAlH_4$ were present when the reduction was started; the remainder was added 0.5 hr. after addition of the aminoketone.

[2] Purified by preparative TLC [silica gel GF_{254}; solvent:petroleum ether:acetone (85:15); R_f=0.30]; m.p. 150-153°.

[3] In this run 1.9 mmoles of $LiAlH_4$ were present when the reduction was started; the remainder was added in two equal portions 1 hr. and 2 hrs. after addition of the aminoketone.

[4] Purified by preparative TLC [silica gel GF_{254}; solvent:chloroform:ethyl acetate: acetone (20:2:1); R_f=0.39]; m.p. 125°.

[5] Purified by preparative TLC [silica gel GF_{254}; solvent:petroleum ether:acetone (85:15); R_f=0.20]; m.p. 123°; the u.v. was identical with that of an authentic sample.

[6] 2-Aminofluoren-9-ol, 66 mg, 25% yield, m.p. 197-199°, was isolated from the reaction mixture. The I.R. was identical with that of an authentic sample.

intermediate, 1-aminofluoren-9-ol, appears to proceed less readily than that of 3-aminofluoren-9-ol (Table 2). In contrast to the reduction of 3- and 1-aminofluoren-9-one the reduction of 2-aminofluoren-9-one by $LiAlH_4$-$AlCl_3$ gave

TABLE 2

THE YIELD OF AMINOFLUORENES IN THE REDUCTION OF AMINOFLUORENOLS BY $LiAlH_4$-$AlCl_3$

Aminofluorenol	mmoles	$LiAlH_4$ (mmoles)	$AlCl_3$ (mmoles)	Aminofluorene (mmoles)	%	Aminofluorenol recovered (mmoles)	%
3-aminofluoren-9-ol	1.02	3.0	4.1	0.57[1]	56	-	-
1-aminofluoren-9-ol	1.02	3.0	4.2	0.39[2]	38	0.49	48
2-aminofluoren-9-ol	1.01	3.0	4.1	0.05[3]	5.5	0.64	63

[1]Reduction as described in the text. An aliquot of a methanolic solution of the product was chromatographed [silica gel GF_{254}; solvent:petroleum ether:acetone (85:15)]. 3-Aminofluorene, R_f=0.39, was extracted from the gel with MeOH and the amount of amine was determined spectrophotometrically (λ_{max}^{MeOH} 240 nm, ε=18,100); only small amounts of 3-aminofluoren-9-ol were seen on the chromatogram.

[2]Reduction as described in the text. An aliquot of methanolic solution of the product was chromatographed [silica gel GF_{254}; solvent:chloroform:methanol (9:1)]. 1-Aminofluorene, R_f=0.46, and 1-aminofluoren-9-ol, R_f=0.14, were extracted from the gel with methanol and the amount of each compound was determined spectrophotometrically (1-aminofluorene, λ_{max}^{MeOH} 250 nm; ε=18,100; 1-aminofluoren-9-ol, λ_{max}^{MeOH} 260 nm, ε=14,100). No 1-aminofluorene was detected chromatographically when $AlCl_3$ was omitted.

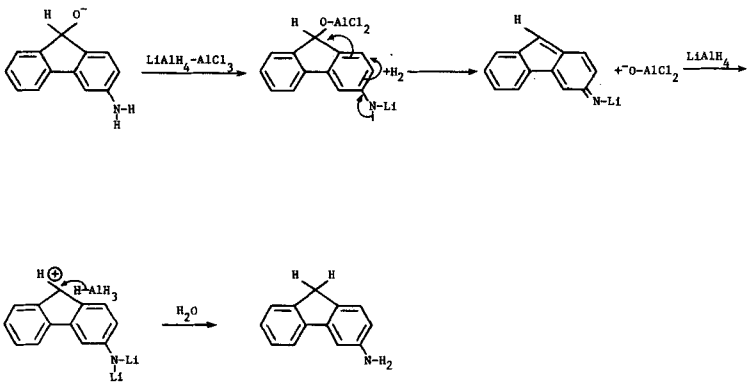
[3]Reduction as described in the text. An aliquot of a methanolic solution of the product was chromatographed [silica gel GF_{254}; solvent:chloroform:methanol (97:3)]. 2-Aminofluorene, R_f=0.52, and 2-aminofluoren-9-ol, R_f=0.13, were extracted from the gel with MeOH and the amounts of each compound were determined spectrophotometrically (2-aminofluorene, λ_{max}^{MeOH} 287 nm; ε=21,000; 2-aminofluoren-9-ol, λ_{max}^{MeOH} 297 nm, ε=22,700).

negligible amounts of amine. The major product of the reaction was 2-aminofluoren-9-ol suggesting that $LiAlH_4$ failed to reduce the aminoalcohol. This conclusion proved to be correct when it was shown in separate experiments (Table 2) that reaction of $LiAlH_4$-$AlCl_3$ with 2-aminofluoren-9-ol yielded only insignificant amounts of amine. As expected, 3- and 1-aminofluoren-9-ol were reduced to the respective amines.

It has previously been reported that fluoren-9-one is reduced by $LiAlH_4$-$AlCl_3$ to fluoren-9-ol in 90-94% yield[8]. We repeated the reduction of fluoren-9-one and likewise obtained only the alcohol in nearly quantitative yield. It is evident that the amino group attached to carbon atoms 3 or 1 facilitates reduction of the alcohol. The role of the amino group may be rationalized by the mechanism of Figure 2. Removal of one of the protons of the amino group leads to a transient imine involving carbon atom-9 and results in the hydrogenolysis of the benzylic system. A conjugated system involving carbon atom-9 and the substituted aromatic ring can, however, only be written when the amino

Figure 2

Reaction Mechanism for the Reduction of 3-Aminofluoren-9-ol to Fluoren-3-amine with $LiAlH_4$-$AlCl_3$



group is linked to carbon atoms 3 or 1 of the fluorene system. In the case of 2-aminofluoren-9-ol conjugation of the imine would extend into the unsubstituted adjacent phenyl ring rather than into the methylene bridge thus precluding hydrogenolysis.

Although the synthesis of the ^{3}H-labeled intermediates presents no difficulties, it should be noted that the catalytic reduction of nitro-[9-^{3}H]fluorene is the only route leading to the labeled hydroxamic acid with retention of ^{3}H. Reduction of 3-nitro-[9-^{3}H]fluorene with $(NH_4)_2S$ to N-fluoren-3-ylhydroxylamine[9,10] and subsequent acetylation of the hydroxylamine[10] which is the alternative route for the preparation of N-fluoren-3-ylacetohydroxamic acid is not feasible because the reduction proceeds in a medium saturated with NH_3 and >99% exchange of ^{3}H has been encountered under these conditions. We have also commonly observed ^{3}H exchange in the catalytic reduction of 3-nitro-[9-^{3}H]fluorene to N-[9-^{3}H]fluoren-3-ylacetohydroxamic acid as indicated by a decrease (~35%) of the specific radioactivity of the ^{3}H-hydroxamic acid versus that of the labeled nitro compound. Since loss of ^{3}H from carbon atom-9 is promoted by base, as shown above, the ^{3}H exchange in this reaction is most probably due to the presence of triethylamine in the reaction mixture. We have recently found that the loss of ^{3}H may be held to approximately 20% by replacement of triethylamine with an equal amount of the weaker base, dimethylaniline.

The exchange of ^{3}H from N-[9-^{3}H]fluoren-3-ylacetohydroxamic acid or of N-[9-^{3}H]fluoren-3-ylacetamide at pH 7.4, i.e. under physiological conditions, was negligible as shown by an experiment in which the ^{3}H-hydroxamic acid was kept in Tris buffer, pH 7.4 at 37°. After 1 hr. the mixture was distilled at reduced pressure and at 26°. Radioassay of the distillate indicated that <1.5% of the radioactivity of the N-[9-^{3}H]fluoren-3-ylacetohydroxamic acid was distillable. In the case of N-[9-^{3}H]fluoren-3-ylacetamide the exchange of ^{3}H in phosphate buffer, pH 7.4, measured in the same way was <0.5%. The exchange of ^{3}H of N-[9-^{3}H]fluoren-3-ylacetohydroxamic acid <u>in vivo</u> was

estimated by determination of the radioactivity distillable from rat-urine collected for 72 hrs. after injection of the ^{3}H-hydroxamic acid. The distillable radioactivity amounted to 12.8 ± 0.1% of the urinary radioactivity (average of 2 experiments). The relatively small ^{3}H exchange in vivo may have occurred in the urine. There is also the possibility that the replacement of the hydrogen of the methylene carbon is a minor metabolic reaction in the rat that was not detected heretofore.

EXPERIMENTAL

Melting points were taken on a Fisher-Johns apparatus and are uncorrected. U.V. and i.r. spectra were recorded with a Beckman DK-2 recording spectrophotometer and a Beckman IR-10 spectrophotometer, respectively. U.V. absorbance measurements were made with a Beckman DU spectrophotometer and read on a Gilford digital absorbance meter. Analytical and preparative TLC was carried out on 20 x 20 cm glass plates coated with silica gel GF_{254} (Brinkmann Instruments, Inc., Westbury, N.Y.). The thickness of the gel was 0.25 mm for analytical TLC and 1 mm for preparative TLC. The radioactivity of tritiated compounds was determined in a toluene-based scintillator[11] with a liquid scintillation spectrometer (Packard Tricarb Model 3375). The counting efficiency was 28-30%. All samples were counted with an error of <5% and corrections were made for quenching. Radiochromatograms were scanned with a TLC scanner (Brinkmann/Berthold Model LB 2721, Brinkmann Instruments, Inc., Westbury, N.Y.).

1-Aminofluoren-9-one, 2-aminofluoren-9-one and 2-aminofluoren-9-ol were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. 3-Aminofluoren-9-one, m.p. 146-150° [reported: 142-146°][12] was prepared by reduction of 3-nitrofluoren-9-one (Aldrich Chemical Co.) with ammonium sulfide[12] or with hydrazine hydrate and Raney Nickel[13,14].

3-Aminofluoren-9-one tosylhydrazone

A mixture of 3-aminofluoren-9-one (0.24 g, 1.2 mmole), p-toluenesulfonylhydrazide (0.26 g, 1.4 mmole) and concentrated HCl (0.5 ml) in 5 ml 95% ethanol was heated briefly on the steam bath. The hydrochloride of 3-aminofluoren-9-one tosylhydrazone, m.p. 182-187° (0.389 g, 75% yield) precipitated and was recrystallized twice from ethanol-diethyl ether without change of m.p. Calculated for $C_{20}H_{18}N_3OSCl$: C, 60.06; H, 4.55; N, 10.51. Found: C, 59.80; H, 4.66; N, 10.69. The hydrochloride was stirred in 50 ml 5% $NaHCO_3$ for 3 hrs. and the hydrazone was recrystallized twice from ethanol-water, m.p. 171-173°. Calculated for $C_{20}H_{17}N_3O_2S$: C, 66.11; H, 4.72; N, 11.57. Found: C, 66.16; H, 4.75; N, 11.79.

1-Aminofluoren-9-one tosylhydrazone

1-Aminofluoren-9-one (1.50 g, 7.7 mmole), p-toluenesulfonylhydrazide (2.12 g, 11.4 mmole) and concentrated HCl (1.3 ml) in 30 ml 95% ethanol was refluxed for 6 hrs. The mixture was cooled in ice and the precipitate (1.45 g), melting from 177-187°, was collected. A portion of the crude product (0.92 g) was purified by preparative TLC with benzene:methanol (99:1) as a solvent. The title compound, R_f=0.21, was extracted from the gel with methanol and recrystallized twice from 95% ethanol; 0.156 g, m.p. 188-190°. Calculated for $C_{20}H_{17}N_3O_2S$: C, 66.11; H, 4.72; N, 11.57. Found: C, 65.92; H, 4.85; N, 11.42

2-Aminofluoren-9-one tosylhydrazone

A solution of 2-aminofluoren-9-one (0.56 g, 2.9 mmole), p-toluenesulfonylhydrazide (0.60 g, 3.2 mmole) in 95% ethanol (30 ml) and glacial acetic acid (0.70 ml) was refluxed for 3 hours. The mixture was cooled in ice whereupon the title compound, m.p. 191-193°, precipitated; 0.66 g, 63%. For analysis the compound was recrystallized from ethanol-water, m.p. 195-196°. Calculated for $C_{20}H_{17}N_3O_2S$: C, 66.11; H, 4.72; N, 11.57. Found: C, 66.17; H, 4.97; N, 11.63.

3-Aminofluoren-9-ol

This compound, m.p. 157-159° [reported: 146-147°][15], was prepared in quantitative yield by reduction of 3-aminofluoren-9-one with $NaBH_4$. TLC of the product on silica gel GF_{254} with chloroform:methanol (97:3) as solvent gave a single spot, R_f=0.20. Calculated for $C_{13}H_{11}ON$: C, 79.16; H, 5.62; N, 7.10. Found: C, 79.29; H, 5.37; N, 7.29.

1-Aminofluoren-9-ol

To a stirred solution of 1-aminofluoren-9-one (0.30 g, 1.54 mmole) in 25 ml of MeOH was added $NaBH_4$ (0.545 g, 14.4 mmole) in portions of 50, 90, 201 and 204 mg over a period of 0.5 hr. After the last addition, the initially brown solution became colorless. The product, m.p. 179-181°, 0.236 g, 78% yield, precipitated upon dilution of the reaction mixture with 100 ml of H_2O. TLC on silica gel GF_{254} with chloroform:methanol (97:3) gave a single spot, R_f=0.19. For analysis, the compound was recrystallized thrice from ethanol-water, m.p. 183-185°; ν_{max}^{KBR} 3400 (-OH), 3320, 3200 ($-NH_2$) cm^{-1}. Calculated for $C_{13}H_{11}ON$: C, 79.16; H, 5.62; N, 7.10. Found: C, 79.29; H, 5.70; N, 6.92.

Reduction of the tosylhydrazones of 2-amino-, 3-amino- and 1-aminofluoren-9-one with $LiAlH_4$

The reduction of the tosylhydrazone of 2-aminofluoren-9-one (0.80 g, 2.2 mmole) in 20 ml THF with an excess of $LiAlH_4$ (1.62 g, 43 mmole) comparable to that used in the reduction of steroidal ketones[6] was carried out in a nitrogen atmosphere for 24 hrs. At this time TLC showed the presence of 6 compounds. Following decomposition of excess $LiAlH_4$ with 10% NH_4Cl the reaction mixture was extracted with diethyl ether. The ether was washed with water and then extracted with 1N HCl. The extract was made basic with 1N KOH and the resulting precipitate was purified by preparative TLC on silica gel GF_{254} with chloroform:methanol (9:1) as solvent. The compound migrating with the mobility of authentic 2-aminofluorene, R_f=0.65, was eluted from the gel with MeOH; 0.028 g, 7% yield. Its u.v. spectrum matched that of authentic 2-aminofluorene. 3- or 1-Aminofluorene

could not be isolated when the tosylhydrazones of 3- or 1-aminofluoren-9-one were reacted with $LiAlH_4$ under the same conditions.

Reduction of 1-Aminofluoren-9-one with $LiAlH_4$

A solution of 1-aminofluoren-9-one (0.54 g, 2.8 mmole) in THF (15 ml) was added dropwise to $LiAlH_4$ (0.60 g, 16 mmole) in THF (100 ml). The mixture was refluxed with stirring for 5 hrs. TLC of an aliquot of the reaction mixture with $CHCl_3$: MeOH (97:3) as a solvent showed no fluoren-1-amine. Fresh $LiAlH_4$ (0.30 g, 7.9 mmoles) was added and refluxing was continued for 43 hrs. when TLC showed a small amount of amine. Addition of $LiAlH_4$ (0.20 g, 5.2 mmole) was repeated and refluxing was continued for a total of 72 hrs. Excess $LiAlH_4$ was decomposed by successive additions of moist diethyl ether (100 ml) and dilute NaOH. The ether was washed with water (2 x 100 ml), dried (anhydrous Na_2SO_4) and evaporated. The residue was subjected to preparative TLC with chloroform:ethyl acetate:acetone (20:2:1) as a solvent. The fluoren-1-amine, R_f=0.48, was eluted from the gel with hot EtOH; m.p. 119-122 [reported: 124-125°](16), 0.03 g, 6% yield.

Reduction of 3-amino, 1-amino- and 2-aminofluoren-9-one with $LiAlH_4$-$AlCl_3$

To a solution of 3-aminofluoren-9-one (0.26 g, 1.3 mmole) in dry diethyl ether (125 ml) was added $AlCl_3$ (0.42 g, 3.2 mmole). The bright-red solution was added dropwise to a stirred and refluxing solution of $LiAlH_4$ (0.18 g, 4.8 mmole) and $AlCl_3$ (0.20 g, 1.5 mmole) in diethyl ether (100 ml). During addition of the aminoketone the reaction vessel was flushed continuously with nitrogen. The solution containing the aminoketone was decolorized instantly upon contact with the $LiAlH_4$-$AlCl_3$. After addition had been completed, the mixture was refluxed with stirring for 2.5 hrs. It was then cooled in ice and excess $LiAlH_4$ was decomposed by addition of water (100 ml). The ether was washed with water (2 x 50 ml) and dried (anhydrous Na_2SO_4). The solvent was evaporated and the

residue was purified by preparative TLC with petroleum ether:acetone (85:15) as solvent. The compound migrating with the mobility of marker fluoren-3-amine, R_f=0.36, was obtained by continuous extraction of the gel for 12 hrs. with MeOH; m.p. 150-154° [reported: 152-153°][17], 0.15 g, 64% yield. The u.v. spectrum of the isolated product matched that of authentic fluoren-3-amine.

The reduction of 1-aminofluoren-9-one was carried out essentially by the above procedure except that only 1/3 of the total amount of $LiAlH_4$ used was present at the start of the reaction. The remainder was added in 2 equal portions 1 hr. and 2 hrs. after addition of the aminoketone had been completed. Purification of the product by TLC with chloroform:ethyl acetate:acetone (20:2:1) gave fluoren-1-amine (R_f=0.39), m.p. 125°, 0.12 g, 44% yield. In addition, 1-aminofluoren-9-ol, (R_f=0.14), m.p. 181°, was isolated; 0.065 g, 21% yield. The identity of the isolated products was confirmed by the u.v. and i.r. spectra.

The reduction of 2-aminofluoren-9-one (0.25 g, 1.3 mmole) and isolation of the reaction products was carried out as described for 3-aminofluoren-9-one. Purification by TLC with petroleum ether:acetone (85:15) as a solvent gave fluoren-2-amine, 0.006 g, 3% yield. The u.v. spectrum of the isolated compound matched that of authentic fluoren-2-amine. In another run the material remaining at the origin after TLC of the crude reaction products was extracted with MeOH and rechromatographed with benzene:ethyl acetate (7:3) as solvent. 2-Aminofluoren-9-ol, m.p. 197-199°, was isolated from the band migrating with R_f=0.17; 0.066 g, 26% yield. The identity of the aminoalcohol was confirmed by comparison of the u.v. and i.r. spectra with those of the authentic compound.

Reduction of 3-Amino, 1-amino- and 2-aminofluoren-9-ol with $LiAlH_4$-$AlCl_3$

To a stirred and refluxing solution of $LiAlH_4$ (0.11 g, 3.0 mmole) and $AlCl_3$ (0.55 g, 4.1 mmole) in dry diethyl ether under nitrogen was added dropwise a bright-yellow solution of 3-aminofluoren-9-ol (0.20 g, 1.0 mmole) in

dry diethyl ether (75 ml). The color of the ethereal solution was discharged instantly upon contact with the reducing mixture. Following addition of the 3-aminofluoren-9-ol the mixture was refluxed with stirring for 2.5 hrs. Analytical TLC of a sample of the reaction mixture on silica gel GF_{254} with petroleum ether:acetone (85:15) showed fluoren-3-amine (R_f=0.39), 3-amino-fluoren-9-ol (R_f=0.14) and a trace of an unidentified product (R_f=0.07). The reaction was discontinued by the decomposition of excess $LiAlH_4$ with water (100 ml). The ether was washed with water and dried. Following evaporation of the ether the residue was dissolved in acetone (100 ml). Triplicate aliquots (0.025 ml x 3) were subjected to analytical TLC with the above solvent. The area of the gel containing fluoren-3-amine (R_f=0.39) was scraped from the plate and the compound was eluted from the gel with MeOH (2 x 2.0 ml). The u.v. spectrum of the solution was identical with that of authentic fluoren-3-amine (λ_{max}^{MeOH} 240,316 nm, with shoulders at 262 and 272 nm). The amount of amine in the MeOH was determined spectrophotometrically (ε_{240} = 18,100) and the total quantity of amine produced in the reduction was calculated from this value (0.10 g, 56% yield).

The reduction of 1-amino- and 2-aminofluoren-9-ol as well as the isolation and spectrophotometric estimation of the respective amines was carried out as described above for 3-aminofluoren-9-ol. The yields of fluoren-1- and fluoren-2-amine were 38 and 5.5%, respectively (Table 2).

N-[9-^{3}H]Fluoren-3-ylacetohydroxamic acid

1. [9-^{3}H]Fluoren-3-amine - To a stirred solution of $LiAl^3H_4$ (0.183 g, 4.8 mmole; specific radioactivity = 1 mCi/mmole) and $AlCl_3$ (0.20 g, 1.5 mmole) in dry diethyl ether refluxing under nitrogen was added dropwise a solution of 3-aminofluoren-9-one (0.27 g, 1.4 mmole) and $AlCl_3$ (0.40 g, 3.0 mmole) in dry diethyl ether (80 ml). After addition had been completed, the stirred solution was refluxed under nitrogen for 2 hrs. The mixture was cooled in ice and excess $LiAl^3H_4$ was decomposed with water (100 ml). [9-^{3}H]Fluoren-3-amine was isolated

and purified by preparative TLC with petroleum ether:acetone (85:15) as described above; m.p. 147-149°, 0.163 g, 63% yield; specific radioactivity = 2.17×10^9 dpm/mmole. Analytical TLC of the compound with benzene:n-hexane (7:3) and scanning of the developed chromatogram showed a single radioactive peak, R_f=0.19.

2. 3-Nitro-[9-^{3}H]fluorene - Oxidation of [9-^{3}H]fluoren-3-amine (0.30 g, 1.7 mmole), specific radioactivity = 1.2×10^9 dpm/mmole, obtained by dissolving 0.163 g of the labeled amine and 0.137 g of unlabeled compound in 25 ml $CHCl_3$ with m-chloroperoxybenzoic acid (0.88 g, 5.1 mmole) in $CHCl_3$ (25 ml) yielded 3-nitro-[9-^{3}H]fluorene, m.p. 102-104° [reported: 105°](18); 0.220 g, 63% yield. TLC of the compound with benzene: n-hexane (8:2) and scanning of the radiochromatogram showed a single radioactive peak, R_f=0.58.

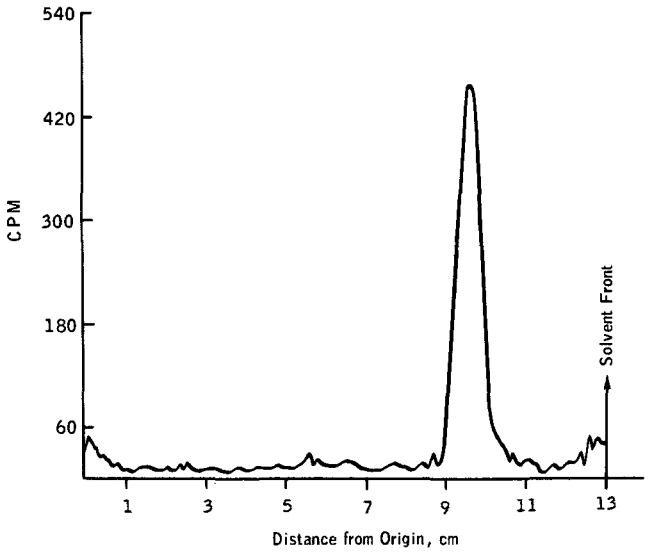
3. N-[9-^{3}H]Fluoren-3-ylacetohydroxamic acid - 3-Nitro-[9-^{3}H]fluorene (0.108 g, 0.5 mmole) in ethyl acetate (25 ml) was hydrogenated with 10% Pd-C (0.020 g) as catalyst in presence of acetic anhydride (0.14 ml, 1.5 mmole) and $(C_2H_5)_3N$ (0.005 ml). After completion of the H_2 up-take the mixture was filtered and the filtrate was stirred for 4 hrs. at room temperature with 7 M NH_4OH (15 ml). The labeled hydroxamic acid was isolated by solvent extraction and the crude product (0.067 g) was recrystallized twice from ethanol-water. The N-[9-^{3}H]fluoren-3-ylacetohydroxamic acid (m.p. 132-134° [reported: 132-134°](5), 0.039 g, 32% yield; specific radioactivity = 7.60×10^8 dpm/mmole gave a single radioactive peak, R_f=0.72, upon chromatography on Eastman Chromagram sheets #6061 and scanning of the radiochromatograms. (Figure 3). The u.v. spectrum of the labeled product was identical with that of authentic N-fluoren-3-ylacetohydroxamic acid.

N-[9-^{3}H]Fluoren-3-ylacetamide

The labeled amide, m.p. 190-192° [reported: 189-190°](16), specific radioactivity = 7.60×10^8 dpm/mmole, was prepared in 43% yield by the reduction of 3-nitro-[9-^{3}H]fluorene with zinc dust, 78% ethanol and $CaCl_2$ followed by acetylation of the resulting amine with acetic anhydride as described previously(19).

Figure 3

Radiochromatogram of N-[9-^{3}H]fluoren-3-ylacetohydroxamic Acid
Solvent: $CHCl_3$:MeOH (95:5)



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