Hyperchromicity (Table II).—Alkaline digestion could not be used for those compounds with a 3-methyluracil residue since this moiety undergoes degradation in alkali.[10] Hyperchromicity was determined by measuring the optical density at the λ_{max} of a solution of the diribonucleoside phosphate at pH 6.0 before and after digestion with purified snake venom diesterase (Worthington Biochemicals). The digestion was carried out as follows. To 2 ml. of an aqueous solution of the diribonucleoside phosphate was added 0.2 ml. of 0.1 *M* Tris buffer (pH 8.2), 0.025 ml. of 0.1 *M* $MgSO_4$ solution, and 0.1 ml. of a freshly prepared enzyme solution (5 mg./ml.). Incubation was carried out at 37° in a jacketed compartment of a Cary Model 14 spectrophotometer and was judged complete when the optical density at λ_{max} remained steady for several hours. In one experiment 5-methyluridylyl-(3'→5')-5-methyluridine was hydrolyzed with 0.5 *N* NaOH and the hyperchromicity measured at pH 6.0 was similar to that obtained by use of the enzyme. In separate experiments the effectiveness of the enzyme treatment was tested on more concentrated samples of each of the diribonucleoside phosphates under similar conditions. The products of the digestion were separated by electrophoresis and in no case could the unchanged diribonucleoside phosphate be detected.

Ribonuclease Treatment.—To the diribonucleoside phosphate (0.2 mg.) in 100 λ of water was added 25 λ of 0.1 *M* Tris buffer solution pH 7.0 and 25 λ of pancreatic ribonuclease solution (1 mg./ml. of Worthington crystalline, Code R), and the solution was incubated at 37° for 15 hr. The reaction mixture was examined by means of electrophoresis.

The following compounds were not hydrolyzed: 3-methyluridylyl-(3'→5')-3-methyluridine, 3-methyluridylyl-(3'→5')-uridine, and 5-methyluridylyl-(2'→5')-5-methyluridine. The following compounds were completely hydroyzed to the 3' nucleotide and nucleoside: uridylyl-(3'→5')-3-methyluridine and 5-methyluridylyl-(3'→5')-5-methyluridine.

When a mixture of equal parts of 5-methyluridylyl-(2'→5')-5-methyluridine and 5-methyluridylyl-(3'→5')-5-methyluridine was subjected to these hydrolytic conditions, no breakdown products were detected.

Acknowledgments.—The authors wish to thank Dr. J. J. Fox for samples of 1-xylofuranosylthymine and 1-arabinofuranosylthymine. They would also like to acknowledge the continued interest and encouragement of Dr. C. A. Nichol in this program. The authors would like to thank the Cancer Chemotherapy National Service Center for a gift of chemicals. This research was partially supported by a grant from the National Cancer Institute of the United States Public Health Service (CA-05697).

Derivatives of Fluorene. XXI. New Halogenofluorenes. II.[1a] Further Potential Antitumor Agents

HSI-LUNG PAN AND T. LLOYD FLETCHER[1b]

Chemistry Research Laboratory of the Department of Surgery, University of Washington School of Medicine, Seattle, Washington 98105

Received February 15, 1965

Further polyhalofluorene derivatives have been found effective against certain tumors in mice, and data are presented for some of the compounds reported earlier.[1a] On the basis of present data, the most active compounds in this group are: N-2-(1,3-dichloro-7-nitrofluorenyl)acetamide (Adenocarcinoma 755), N-2-(7-bromo-1,3,4-trichlorofluorenyl)acetamide (Sarcoma 180), N-2-(1,3,7-trichlorofluorenyl)acetamide (Cloudman melanoma), 1,3-dibromo-7-nitro-2-fluorenamine (Adencarcinoma and Sarcoma), 1,3,7-tribromo-2-fluorenamine (Adenocarcinoma), and N-2-(1,3,4,6,7-pentachlorofluorenyl)acetamide (Sarcoma). The position of the chlorine atom, designated earlier[1a] as 4(?) on the basis of infrared absorption, is confirmed by synthesis. Some compounds we described as 2,3,7-trisubstituted fluorenes are shown to be 2,4,7-derivatives, in agreement with recent reports,[2,3] but still in marked disagreement with an earlier paper.[4] 2,7-Dibromo-, -dichloro-, and -difluorofluorenes and the three corresponding 9-oxofluorenes are all nitrated at the 4-position. Chlorination of N-2-(4-chlorofluorenyl)acetamide, N-2-fluorenylformamide, and ethyl N-2-fluorenylcarbamate, as found earlier[1a] with N-2-fluorenylacetamide, leads to the 1,3,4,7-tetrachloro compounds. 2,3,7-Trichlorofluorene is prepared by three routes. Acetylation of some relatively weak amines by merely shaking at room temperature with glacial acetic acid is described. Bromination of N-2-(7-bromofluorenyl)acetamide with N-bromosuccinimide in dimethylformamide gives the 3-bromo derivative.

Our previous report in this series[1a] included a number of polyhalogenated fluorene derivatives which show antitumor activity in animals. In expanding this area, in a search for further compounds with biological activity, we have first confirmed the supposed 4-position for a number of these substances. In all cases in the previous paper[1a] the position designation 4(?) is, in fact, 4. In addition to this minor point, we wish, in the present paper, to clarify a discrepancy in the literature and, in so doing, correct a position designation in the names of a number of the reported compounds in the previous paper. In brief, eight di- and trichlorofluorene derivatives (shown in Table I), named 2,3,7-substituted fluorenes, are 2,4,7-substituted fluorenes.

TABLE I

No.[a]	Correct name
XIX	9-Bromo-2,4,7-trichlorofluorene
XX	2,4,7-Trichlorofluoren-9-ol
XXI	9-Oxo-2,4,7-trichlorofluorene
LII	4-Amino-2,7-dichlorofluoren-9-ol
LIII	2,7-Dichloro-9-oxo-4-fluorenamine
LXXIII	2,4,7-Trichlorofluorene
In text only	2,7-Dichloro-4-nitrofluorene
In text only	2,7-Dichloro-4-fluorenamine

[a] See ref. 1a, Table II, or text.

As noted,[1a] there are discrepancies apparent in comparing some of our compounds with those of the same name described by Kretov, *et al.*[4] The latter claimed to have obtained the 3-nitro derivative, by nitrating 2,7-dichlorofluorene, and from this 2,3,7-trichloro-

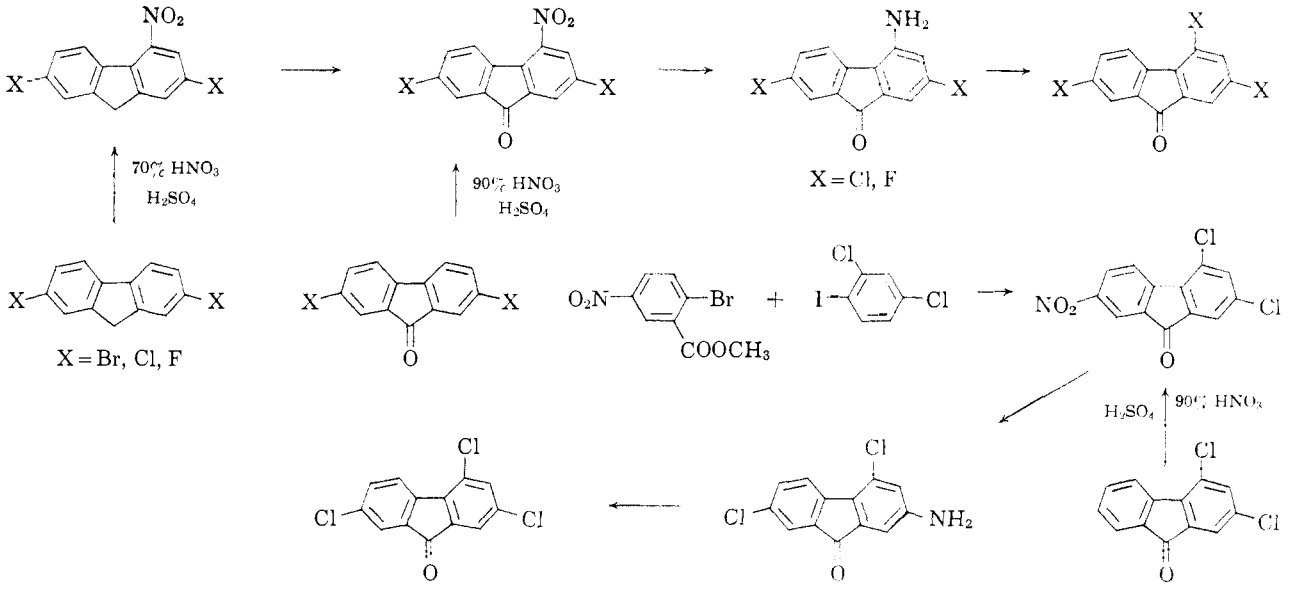
(1) (a) For Halogenofluorenes. I, see H.-L. Pan and T. L. Fletcher, *J. Med. Chem.*, **7**, 31 (1964). (b) Supported in part by a grant (Ca-01744) from the National Cancer Institute, National Institutes of Health, and in part by Research Career Development Award 5-K3-GM-14,991 (T. L. F.).

(2) W. Schidlo and A. Sieglitz, *Chem. Ber.*, **96**, 2595 (1963).

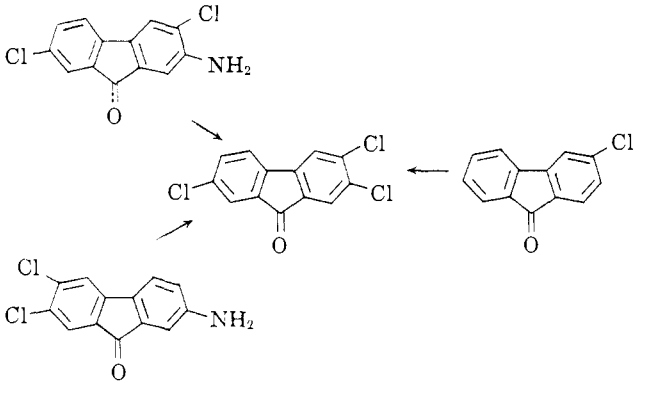
(3) A. Sieglitz, *ibid.*, **97**, 3392 (1964).

(4) In particular see paragraph 2 and footnotes 6–8 in ref. 1a.

SCHEME I



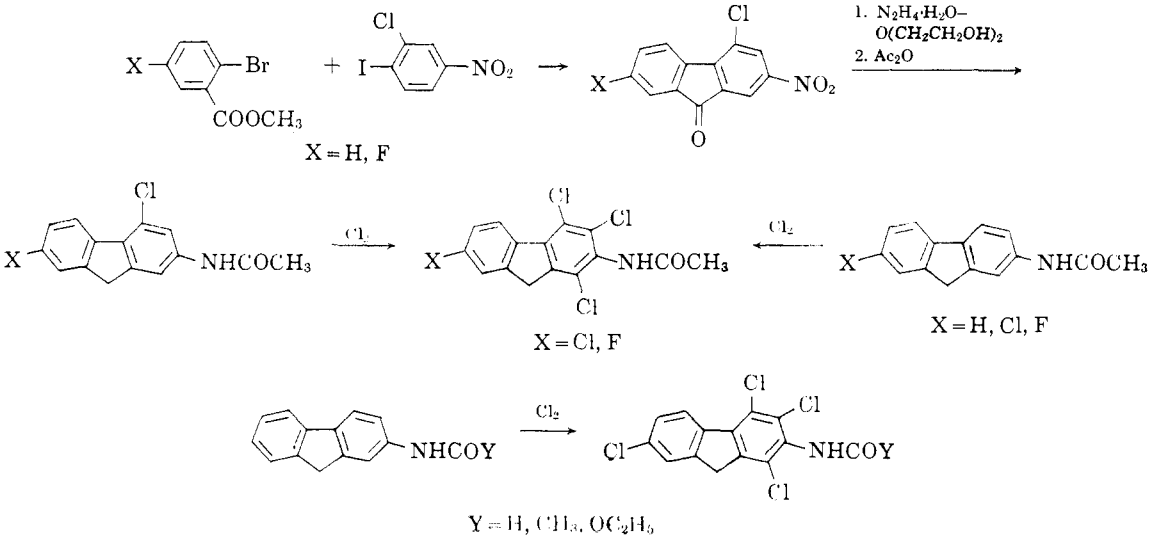
SCHEME II



fluorene were each chlorinated to give a 2,4,7-trisubstituted fluorene. The corrections in Table I and the structures (Scheme I) described in this paper are now consistent and in full agreement with those of Schidlo and Sieglitz[2] and Sieglitz.[3] We are still in substantial disagreement, however, with Kretov's work, and, on the basis of the details we have seen, we are unable to clarify his results.[4]

Authentic 2,3,7-trichlorofluorene was prepared in three ways. Both 3,7-dichloro-9-oxo-2-fluorenamine and 6,7-dichloro-9-oxo-2-fluorenamine were converted to 2,3,7-trichloro-9-oxofluorene,[3] through a Sandmeyer reaction, and 3-chloro-9-oxofluorene was chlorinated to the same trichloro compound (melting point and infrared spectra) (see Scheme II).

SCHEME III



fluorene. We find, however, that not only 2,7-dichlorofluorene but also the 2,7-dibromo and -difluoro derivatives as well as the corresponding 2,7-dihalo-9-oxofluorenes nitrate in the 4-position. While this work was in progress, further confirmation of the above appeared[2]: in the latter, 4-carboxyfluorene and 4-chloro-

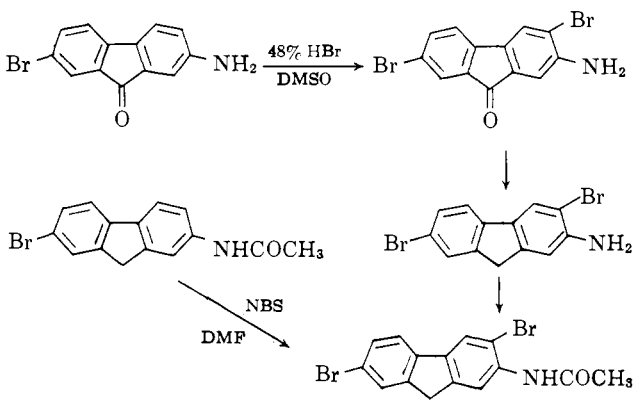
We leaned heavily on infrared spectra[1a] to support our belief that many of our tri-, tetra-, and pentachlorofluorene derivatives were substituted in the 4-position by one of the chlorine atoms. In this report we present chemical evidence that proves this to be true as shown in Scheme III. Synthesis of 2-acetamido-4-chloro-

fluorene and 2-acetamido-4-chloro-7-fluorofluorene followed by chlorination gave the same compounds as we described earlier made by a different route.

Chlorination of 2-formamidofluorene and ethyl N-2-fluorenylcarbamate gives tetrachloro derivatives with the chlorines most likely in the 1-, 3-, 4-, and 7-positions as in the chlorination of 2-acetamidofluorene.[1a] Infrared spectra of these compounds show similar absorptions in the 800–900-cm.$^{-1}$ region, indicating that two adjacent hydrogens are present in these molecules studied.[1a]

Antitumor activities of some of the polyhalogenated fluorenes are shown in Table II. The most active compounds are substituted with chlorine or bromine at the 1- and 3-, or 1-, 3-, and 4-positions and with halo, including fluoro, or nitro at the 7-position of 2-amino- or 2-acetamidofluorene. Whether blocking at both positions *ortho* to the 2-amino group is necessary for activity or for lowering toxicity is not yet known, but we are preparing compounds with only one of the *ortho* positions blocked so that this question can be explored. The first of these compounds, soon to be tested, was made from N-2-(7-bromofluorenyl)acetamide by brominating in dimethylformamide with N-bromosuccinimide.[5] An alternate route is also shown in Scheme IV. Acetylation of two relatively weak amines, by merely shaking at room temperature in glacial acetic acid, is described.

SCHEME IV



One further question worthy of examination, of greater significance than for this series of compounds alone, is the relation between carcinogenicity (a well-established property of 2-acetamidofluorene and some of its derivatives) and tumor inhibition.

Experimental[6]

2,7-Dichloro-4-nitro-9-oxofluorene. A.—2,7-Dichloro-9-oxofluorene[7] (30 g., 0.12 mole) was added portionwise to a stirred mixture of acetic acid (600 ml.) and 90% HNO_3 (600 ml.). The mixture was heated to 50°, then concentrated H_2SO_4 (120 ml.) was introduced at such a rate that the temperature remained below 55°. After all the sulfuric acid had been added, the mixture was stirred at 50–55° for 10 min. and cooled. Water dilution, filtration, and recrystallization from acetone gave 29 g. (82%); m.p. 187–187.5° (lit.[2] m.p. 187°); ν_{max} 1724 (ketone C=O), 840 (two adjacent hydrogens) cm.$^{-1}$.

Anal. Calcd. for $C_{13}H_5Cl_2NO_3$: C, 53.09; H, 1.71. Found: C, 53.25; H, 1.57.

B.—2,7-Dichloro-4-nitrofluorene was oxidized in acetic acid with sodium dichromate giving 98% of material, m.p. 186–187°. The melting point was not depressed when mixed with the product obtained in A.

2,7-Difluoro-4-nitro-9-oxofluorene. A.—2,7-Difluoro-9-oxofluorene[8a] was nitrated as described above giving a 77% yield; m.p. 168.5–169° (acetone); ν_{max} 1724 (ketone C=O), 835 (two adjacent hydrogens) cm.$^{-1}$.

Anal. Calcd. for $C_{13}H_5F_2NO_3$: C, 59.78; H, 1.93; N, 5.36. Found: C, 60.01; H, 1.82; N, 5.69, 5.49.

B.—2,7-Difluoro-4-nitrofluorene was oxidized with dichromate to give a 95% yield of the product in A.

2,7-Difluoro-9-oxo-4-fluorenamine.—The nitro compound was reduced with $SnCl_2 \cdot 2H_2O$ and concentrated HCl giving an 80% yield, m.p. 220–221° (toluene).

Anal. Calcd. for $C_{13}H_7F_2NO$: C, 67.53; H, 3.05. Found: C, 67.79; H, 2.99.

9-Oxo-2,4,7-trifluorofluorene.—The preceding amine was converted to the trifluoro compound by a Schiemann reaction. The melting point and mixture melting point with an authentic sample[8b] were the same and infrared spectra were identical.

2,7-Dibromo-4-nitro-9-oxofluorene. A.—2,7-Dibromo-9-oxofluorene was nitrated in the same manner as the 2,7-dichloro and 2,7-difluoro analogs giving a 10% yield; m.p. and m.m.p. (with the following product) 196–197°; ν_{max} 1724 (ketone C=O), 838 (two adjacent hydrogens) cm.$^{-1}$.

B.—2,7-Dibromo-4-nitrofluorene was oxidized as described for the preceding analogs giving a 98% yield, m.p. 196–197° (toluene–ethanol).

Anal. Calcd. for $C_{13}H_5Br_2NO_3$: N, 3.66. Found: N, 3.52.

N-4-(2,7-Dichloro-9-oxofluorenyl)acetamide.—2,7-Dichloro-9-oxo-4-fluorenamine[1a] (see Table I) was acetylated in the usual manner, m.p. 312–313° (acetic acid).

Anal. Calcd. for $C_{15}H_9Cl_2NO_2$: C, 58.85; H, 2.96; N, 4.58. Found: C, 58.82; H, 2.94; N, 4.69.

2,7-Dichloro-4-nitrofluorene.—2,7-Dichlorofluorene[1a] (25.5 g., 0.11 mole) was suspended in acetic acid (450 ml.). To the stirred suspension, heated at 35°, a mixture of concentrated H_2SO_4 (25 ml.) and HNO_3 (*d* 1.42) (25 ml.) was added dropwise over a period of 20 min. The suspension was stirred for 5 more min., then heated at 70° for 15 min. and cooled. The product was filtered, washed, and dried giving 28 g. (94%), m.p. 180–181°. Recrystallization from acetic acid gave an analytical sample, m.p. 180.5–181° (lit.[2] m.p. 179–180°).

Anal. Calcd. for $C_{13}H_7Cl_2NO_2$: N, 5.00. Found: N, 4.76.

2,7-Difluoro-4-nitrofluorene.—2,7-Difluorofluorene[9] was nitrated as described above giving 87–92% yields, m.p. 136–137° (methanol).

Anal. Calcd. for $C_{13}H_7F_2NO_2$: C, 63.16; H, 2.85; N, 5.67. Found: C, 63.01; H, 2.93; N, 5.61.

2,7-Dibromo-4-nitrofluorene.—2,7-Dibromofluorene[10] was also nitrated (77%), m.p. 194.5–195.5° (toluene–ethanol).

Anal. Calcd. for $C_{13}H_7Br_2NO_2$: C, 42.31; H, 1.90; Br, 43.31; N, 3.80. Found: C, 42.17; H, 1.90; Br, 43.28; N, 3.96.

2,7-Difluoro-4-fluorenamine.—2,7-Difluoro-4-nitrofluorene (2.5 g.) was dissolved in a mixture of toluene (100 ml.) and 95% ethanol (250 ml.) and reduced with 85% hydrazine hydrate (5

(5) Ng. Ph. Buu-Hoï, *Rec. trav. chim.*, **73**, 197 (1954); T. H. Chao and L. P. Cipriani, *J. Org. Chem.*, **26**, 1079 (1961); we are indebted to M. J. Namkung for pointing out this reaction.

(6) All melting points below 250° were taken on a Fisher-Johns block and are corrected to standards. Melting points above 250° were taken with a Hoover capillary melting point apparatus and are uncorrected. Analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y., and by A. Bernhardt, Mülheim (Ruhr). The interpretation of infrared absorption spectra (obtained on a Beckman IR-5, 1.5–2 mg. of substance/300 mg. of KBr) is based on L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1958. All chlorinations were carried out using a Matheson 620 BMV flowmeter.

(7) J. Schmidt and H. Wagner, *Ann.*, **387**, 147 (1912).

(8) (a) Made by M. J. Namkung of this laboratory by sodium dichromate oxidation of 2,7-difluorofluorene[9] to give 70% of pure compound, m.p. 205–206°. *Anal.* Calcd. for $C_{13}H_6F_2O$: C, 72.22; H, 2.80. Found: C, 72.51; H, 2.89. (b) M. J. Namkung, T. L. Fletcher, and W. H. Wetzel, *J. Med. Chem.*, **8**, 551 (1965).

(9) Made by M. J. Namkung from 7-fluoro-2-fluorenamine by a Schiemann decomposition of the diazonium fluoborate, which was prepared in aqueous tetrahydrofuran [see T. L. Fletcher and M. J. Namkung, *Chem. Ind.* (London), 179 (1961)], in boiling xylene (72% over-all). Sublimation at 75° (1 mm.) followed by recrystallization from methanol–water gave the product, m.p. 82–82.5°. S. Berkovic [*Israel J. Chem.*, **1**, 1 (1963)] reports m.p. 83°.

(10) A. Pictet and L. Ramseyer, *Ber.*, **44**, 2486 (1911).

TABLE II

ANTITUMOR ACTIVITY OF CERTAIN POLYHALOGENOFLUORENE DERIVATIVES[a]

Fluorene	Tumor system[b]	Daily dose, mg./kg.	Survivors	Tumor wt., mg. (T/C)	Tumor wt. decrease, % (1 − T/C)	Specificity test[c] Confidence, %	Index
2-Acetamido-1,3-dichloro-7-nitro-[d]	Ca755	200	9/12	94/1411	94		
		100	11/12	113/1411	92		
		50	11/12	129/1411	91		
		25	12/12	127/1411	91		
		12.5	12/12	217/1411	85		
		6.25	12/12	473/1411	67		
		6.25	10/10	220/706	69		
		3.12	10/10	182/706	75		
		1.60	10/10	338/706	53		
		0.80	9/10	227/706	68		
		0.80	9/10	140/1095	88		
		0.40	10/10	349/1095	69		
		0.20	10/10	235/1095	79		
		0.10	10/10	604/1095	45		
		300	8/12	85/1251	94		
		200	9/12	325/1251	75		
						99.7	3.0
2-Acetamido-7-bromo-1,3,4-trichloro-[d]	S180	63	4/6	346/1034	67		
		41	5/6	643/1034	38		
		28	6/6	368/1034	65		
						95.0	2.0
		19	6/6	413/736	44		
		13	6/6	342/736	54		
		9	5/6	514/736	31		
		6	6/6	281/736	62		
						99.7	2.1
2-Acetamido-1,3,4,7-tetrachloro-[d]	Ca755	110	5/10	250/934	73		
		55	8/10	291/934	69		
		27	10/10	813/934	13		
						99.7	3.0
2-Acetamido-1,3,4,6,7-pentachloro-[d]	S180	375	5/6	235/1164	80		
		250	5/6	254/1164	79		
		375	6/6	821/1742	53		
		250	6/6	755/1742	57		
		166	6/6	503/1742	72		
		110	6/6	545/1742	69		
		110	5/6	487/1670	71		
		73	6/6	518/1670	69		
		49	6/6	675/1670	60		
		33	6/6	819/1670	51		
		166	2/6	228/1042	78		
		110	1/6	100/1042	99		
						99.7	1.7
2-Acetamido-1,3,7-trichloro-	S91	200	10/10	307/1158	74[e]		
2-Formamido-1,3,4,7-tetrachloro-	S180	250	6/6	414/1569	74[f]		
2-Amino-1,3-dibromo-7-chloro-	S180	62	5/6	1005/1670	40		
		41	6/6	750/1670	56		
		27	6/6	880/1670	48		
						99.7	2.6
2-Amino-1,3-dibromo-7-nitro-[d]	S180	500	6/6	359/664	46		
		333	6/6	446/664	33		
		222	6/6	429/664	36		
		148	6/6	373/664	44		
						99.7	4.3
	Ca755	110	10/10	364/1699	79		
		55	10/10	497/1699	71		
		27.5	10/10	792/1699	54		
		200	7/10	112/1392	92		
		100	9/10	268/1392	81		
		50	10/10	173/1392	88		
		25	10/10	101/1392	93		

Fluorene	Tumor system[b]	Daily dose, mg./kg.	Survivors	Tumor wt., mg. (T/C)	Tumor wt. decrease, % (1 − T/C)	Specificity test[c] Confidence, %	Index
		12	7/10	324/1436	78		
		6	7/10	619/1436	57		
		3	9/10	907/1436	37		
		1.5	10/10	1221/1436	15		
						99.7	2.0
2-Amino-1,3-dibromo-7-phenylazo-	S180	125	6/6	112/520	78[g]		
2-Amino-1,3,7-tribromo-[d]	Ca755	1920	10/10	414/957	57		
		1920	9/10	195/957	80		
		960	10/10	180/957	82		
		30	10/10	238/957	76		
		15	10/10	315/957	68		
		120	10/10	459/957	53		
		60	10/10	368/957	62		
		30	10/10	397/957	59		
						99.7	2.0
		960	10/10	502/1596	69		
		480	10/10	420/1596	74		
		240	10/10	709/1596	56		
		120	10/10	900/1596	44		
		480	10/10	256/1596	84		
		240	10/10	265/1596	84		
		120	10/10	273/1596	83		
		60	10/10	394/1596	76		
		960	9/10	413/1596	75		
		480	9/10	392/1596	76		
		240	10/10	377/1596	77		
		120	9/10	423/1596	74		
						95.0	1.6
2,7-Diamino-1,3-dibromo-	S180	500	6/6	135/520	75[g]		
2-Trifluoroacetamido-3-bromo-7-nitro-9-oxo-[d]	S91	200	8/10	121/510	77[h]		
9-Oxo-2,4,7-trichloro-[d]	S180	500	4/6	175/770	78		
		333	6/6	197/770	75		
		222	6/6	238/770	70		
		333	5/6	458/1395	68		
		222	6/6	620/1395	56		
		149	6/6	828/1395	41		
		98	6/6	625/1395	56		
		147	6/6	333/906	64		
		98	6/6	454/906	50		
		66	5/6	591/906	35		
		43	6/6	692/906	24		
						99.7	2.2
2-Amino-1,3-dibromo-7-acetamido-	S180	125	5/6	20/520	97[g]		

[a] The screening data in this table were kindly supplied by the Cancer Chemotherapy National Service Center, National Institutes of Health, Bethesda, Md. Assays were performed according to specifications established by CCNSC as reported in *Cancer Chemotherapy Rept.*, **25**, 1 (1962). [b] Sarcoma 180 was tested in random bred Albino mice; Adenocarcinoma 755 and Cloudman Melanoma S91 were tested in BDF_1 mice. [c] See footnote *d* in Table III of ref. 1a. [d] This is an extension of data reported earlier.[1a] [e] Passed stage 3 of sequential testing. [f] Activity confirmed. [g] Passed stage 2 of sequential testing. [h] Confirmation test.

ml.) and a small amount of Raney nickel[11] to give a quantitative yield of amine. Recrystallization from ethanol–water gave an analytical sample, m.p. 124.5–125.5°.

Anal. Calcd. for $C_{13}H_9F_2N$: C, 71.88; H, 4.18; N, 6.45. Found: C, 71.74; H, 4.26; N, 6.64.

2,7-Dibromo-4-fluorenamine.—2,7-Dibromo-4-nitrofluorene (7.4 g.) was reduced in the same way giving 6.7 g. (99%), m.p. 189.5–190.5° (ethanol).

Anal. Calcd. for $C_{13}H_9Br_2N$: C, 46.05; H, 2.68; N, 4.13. Found: C, 45.84; H, 2.64; N, 4.34.

N-Acetyl derivative had m.p. 267.5–268° (ethanol).

Anal. Calcd. for $C_{15}H_{11}Br_2NO$: C, 47.28; H, 2.91; N, 3.68. Found: C, 46.87; H, 2.94; N, 3.89.

2,7-Dibromo-9-oxo-4-fluorenamine.—2,7-Dibromo-4-nitro-9-oxofluorene was reduced with $SnCl_2 \cdot 2H_2O$ and concentrated HCl giving a 90% yield, m.p. 253.5–254.5° (toluene).

Anal. Calcd. for $C_{13}H_7Br_2NO$: C, 44.23; H, 2.00; N, 3.97. Found: C, 44.42; H, 1.85; N, 4.25.

N-Acetyl derivative had m.p. 332–333° (toluene).

Anal. Calcd. for $C_{15}H_9Br_2NO_2$: C, 45.60; H, 2.30; Br, 40.46; N, 3.55. Found: C, 45.41; H, 2.62; Br, 40.41; N, 3.70.

2,4-Dichloro-7-nitro-9-oxofluorene. A.—This ketone was synthesized from methyl 2-bromo-5-nitrobenzoate[12] and 2,4-dichloroiodobenzene by an Ullmann procedure as described below, giving a 23% yield; m.p. 230–230.5° (benzene); ν_{max} 1724 (ketone C=O), 844 (two adjacent hydrogens) $cm.^{-1}$.

Anal. Calcd. for $C_{13}H_5Cl_2NO_3$: C, 53.09; H, 1.71; Cl, 24.11; N, 4.76. Found: C, 53.20, 53.19; H, 1.81, 1.66; Cl, 24.04; N, 4.92.

B.—2,4-Dichloro-9-oxofluorene (1 g.) was added in several portions to a mixture of 90% HNO_3 (5 ml.) and acetic acid (5 ml.) at 35°. Concentrated H_2SO_4 (1 ml.) was then added slowly with the temperature held at 40–45°. After 5 min. of stirring

(11) T. L. Fletcher and M. J. Namkung, *J. Org. Chem.*, **23**, 680 (1958).

(12) A. E. Holleman and B. R. deBruyn, *Rec. trav. chim.*, **20**, 206 (1901); R. E. Buckles, R. Filler, and L. Hilfman, *J. Org. Chem.*, **17**, 233 (1952).

the mixture was cooled and diluted with ice–water, and the product was isolated. Recrystallization from toluene gave 0.7 g. (58%), m.p. and m.m.p. (with the product in A) 230–230.5°. The infrared spectra of both samples were identical.

2,4-Dichloro-9-oxo-7-fluorenamine.—2,4-Dichloro-7-nitro-9-oxofluorene was reduced with $SnCl_2 \cdot 2H_2O$ in concentrated HCl giving a 99% yield of amine, m.p. 260.5–261.5° (toluene).

Anal. Calcd. for $C_{13}H_7Cl_2NO$: C, 59.12; H, 2.67; N, 5.30. Found: C, 58.88; H, 2.76; N, 5.60.

9-Oxo-2,4,7-trichlorofluorene.[1a] (see Table I) **A.**—The foregoing amine (1.3 g.) was diazotized at 0–5° in a mixture of concentrated HCl (100 ml.) and water (50 ml.) with $NaNO_2$ (0.7 g.). The diazonium salt was then treated in a hot-water bath with an excess of CuCl giving 1.4 g. of a crude product which was chromatographed in benzene through an alumina column and recrystallized from acetone giving 0.8 g. (57%), m.p. 183.5–184° [lit. m.p. 183.5–184° [1a] (see Table I), 178–179°[2]].

B.—2,7-Dichloro-9-oxo-4-fluorenamine[1a] (see Table I) was converted to the trichloro compound in the same way in 68% yield; melting point and mixture melting point were the same, and infrared spectra of this product and that obtained in A were identical.

3,7-Dichloro-9-oxo-2-fluorenamine.—N-2-(3,7-Dichloro-9-oxofluorenyl)acetamide[1a] was hydrolyzed by refluxing for 4 hr. in a mixture of concentrated HCl and absolute ethanol, m.p. 249–249.5° (toluene).

Anal. Calcd. for $C_{13}H_7Cl_2NO$: N, 5.30. Found: N, 5.29.

9-Oxo-2,3,7-trichlorofluorene. A.—3,7-Dichloro-9-oxo-2-fluorenamine (1.3 g.) was diazotized at room temperature in a mixture of acetic acid (20 ml.) and concentrated H_2SO_4 (10 ml.) with nitrosyl sulfuric acid, prepared from $NaNO_2$ (0.8 g.) and H_2SO_4 (5 ml.). After 1 hr. of stirring, the mixture was poured onto ice (50 g.) and treated with a solution of a slight excess of CuCl in dilute HCl, stirred at 50–55° for 30 min., and diluted with NH_4OH. The precipitate was chromatographed twice (benzene) on alumina giving 0.4 g. (30%). The product and the compound obtained in B were identical as shown by melting point, mixture melting point, and infrared spectra.

B.—6,7-Dichloro-9-oxo-2-fluorenamine[1a] (2.6 g., 0.01 mole) was diazotized at 0–5° with $NaNO_2$ (1.4 g.) in 25% HCl (150 ml.). To this was added a solution of CuCl (excess) in HCl, and the mixture was heated on a water bath for 0.5 hr. and then diluted with water. The crude product was chromatographed (benzene) on alumina giving 1.9 g. (68%); m.p. 215.5–216.5° (lit.[3] m.p. 214.5–215°); ν_{max} 1710 (ketone C=O), 837 (two adjacent hydrogens) cm.$^{-1}$.

Anal. Calcd. for $C_{13}H_5Cl_3O$: C, 55.07; H, 1.78; Cl, 37.51. Found: C, 55.35; H, 1.81; Cl, 37.22.

C.—3-Chloro-9-oxofluorene[13] (2.2 g., 0.01 mole) was chlorinated at 95–100° in acetic acid (200 ml.) containing $FeCl_3$ (2 g.) and water (50 ml.), by bubbling chlorine through the solution first at a rate of 300 ml./min. for 0.5 hr. then at 50 ml./min. for 3 hr. The mixture was cooled and the product was filtered off and recrystallized from benzene giving 1.8 g. (63%) of product, identical with those in A and B (infrared spectra, melting point, and mixture melting point).

4-Chloro-2-nitro-9-oxofluorene.—Copper powder (150 g.; Type 450A, Metals Disintegrating Co., Elizabeth, N. J.) was added portionwise over a period of 40 min. to a rapidly stirred mixture of methyl 2-bromobenzoate (172 g., 0.8 mole) and 3-chloro-4-iodonitrobenzene[14] (113.6 g., 0.4 mole) at 210–215°. After stirring at the same temperature for 20 min., the reaction mixture was cooled and extracted with boiling ligroin (*d* 0.68–0.70). The oil (174 g.) obtained from the ligroin extract was refluxed for 14 hr. in a mixture of acetic acid (1 l.), concentrated H_2SO_4 (0.5 l.), and water (0.25 l.), and diluted with ice–water. The solid thus obtained was refluxed for 2 hr. in 15% Na_2CO_3 (1.2 l.), cooled, and filtered. The filtrate was shaken with chloroform, the aqueous layer was acidified with concentrated HCl and the precipitate was filtered off and thoroughly dried (101 g.). It was then thoroughly mixed with polyphosphoric acid (900 g.) and heated at 150–160° (oven) for 2 hr. and poured onto ice. The solid was filtered off, washed, and extracted with hot 15% Na_2CO_3 (1 l.) and the alkali-insoluble product was recrystallized from toluene giving 37.8 g. (36%), m.p. 209–209.5°.

Anal. Calcd. for $C_{13}H_6ClNO_3$: C, 60.13; H, 2.33; Cl, 13.66; N, 5.39. Found: C, 60.36; H, 2.33; Cl, 13.62; N, 5.65.

4-Chloro-7-fluoro-2-nitro-9-oxofluorene.—This compound was synthesized from 3-chloro-4-iodonitrobenzene and methyl 2-bromo-5-fluorobenzoate,[15] in the manner described above, giving a 20% yield; m.p. 222–223° (benzene); ν_{max} 1724 (ketone C=O), 800 (two adjacent hydrogens) cm.$^{-1}$.

Anal. Calcd. for $C_{13}H_5ClFNO_3$: Cl, 12.77; N, 5.05. Found: Cl, 12.89; N, 5.03.

4-Chloro-9-oxo-2-fluorenamine.—A mixture of 4-chloro-2-nitro-9-oxofluorene (13 g., 0.05 mole), $SnCl_2 \cdot 2H_2O$ (60 g.), concentrated HCl (100 ml.), and 95% ethanol (30 ml.) was gently boiled for 30 min., cooled, and stirred into 2 *N* NaOH (1 l.), and the amine was isolated, 11.5 g. (100%), m.p. 244–246°. Recrystallization from toluene gave an analytical sample, m.p. 246–247°.

Anal. Calcd. for $C_{13}H_8ClNO$: C, 67.99; H, 3.51; N, 6.10. Found: C, 68.03; H, 3.68; N, 6.17.

N-Acetyl derivative had m.p. 262.5–263.5° (acetic acid–toluene).

Anal. Calcd. for $C_{15}H_{10}ClNO_2$: C, 66.31; H, 3.71; N, 5.16. Found: C, 66.24; H, 3.76; N, 5.35.

4-Chloro-2-fluorenamine.—4-Chloro-2-nitro-9-oxofluorene (13 g., 0.05 mole) was reduced[16] by refluxing for 22 hr. in a mixture of 2,2'-oxydiethanol (750 ml.) and 85% hydrazine hydrate (75 ml.), giving 9.9 g. (92%), m.p. 113–114° (ethanol).

Anal. Calcd. for $C_{13}H_{10}ClN$: C, 72.39; H, 4.67; Cl, 16.44; N, 6.49. Found: C, 72.61; H, 4.64; Cl, 16.18; N, 6.25.

N-Acetyl derivative had m.p. 243.5–244°.

Anal. Calcd. for $C_{15}H_{12}ClNO$: C, 69.91; H, 4.69; Cl, 13.76; N, 5.44. Found: C, 69.89; H, 4.59; Cl, 13.68; N, 5.55.

4-Chloro-7-fluoro-2-fluorenamine.—4-Chloro-7-fluoro-2-nitro-9-oxofluorene was reduced as described above[16] giving a 91% yield, m.p. 142.5–143.5° (methanol).

Anal. Calcd. for $C_{13}H_9ClFN$: C, 66.82; H, 3.88; N, 5.99. Found: C, 67.06; H, 3.91; N, 5.94.

N-Acetyl derivative had m.p. 242–243° (methanol).

Anal. Calcd. for $C_{15}H_{11}ClFNO$: C, 65.34; H, 4.02; N, 5.08. Found: C, 65.24; H, 3.99; N, 5.16.

N-2-(1,3,4,7-Tetrachlorofluorenyl)acetamide.[17]—N-2-(4-Chlorofluorenyl)acetamide (2.6 g., 0.01 mole) was suspended in warm glacial acetic acid (350 ml.) containing some anhydrous $FeCl_3$ (~0.5 g.). Chlorine was then bubbled through the stirred suspension at about 150 ml./min. (measured by means of a Matheson 620 BMV flowmeter) for 30 min. while the temperature was maintained at 55–60°. After 1 hr. of stirring at this temperature, the solution was cooled and diluted with water, and the product was isolated giving 1.5 g. (42%), m.p. 281.5–282.5°. The melting point was not depressed when mixed with the compound prepared previously[17] and the infrared spectra of the two samples were identical; ν_{max} 3247 (amide N–H), 1672 (amide I), 820 (two adjacent hydrogens) cm.$^{-1}$.

N-2-(7-Fluoro-1,3,4-trichlorofluorenyl)acetamide.[18]—N-2-(4-chloro-7-fluorofluorenyl)acetamide (1.38 g., 5 mmoles) was dissolved in glacial acetic acid (65 ml.) containing anhydrous $FeCl_3$ (~0.2 g.). The mixture was stirred at 50–60° while chlorine was added at about 100 ml./min. for 1 hr. After further stirring at the same temperature for another hour, the reaction mixture was diluted with water. The solid was recrystallized from 95% ethanol giving 1 g. (59%), m.p. 276–277°. The mixture melting point with the compound prepared from chlorination of N-2-(7-fluorofluorenyl)acetamide[18] was not depressed and the infrared spectra of the two compounds were identical.

N-2-(1,3,4,7-Tetrachlorofluorenyl)formamide.—N-2-Fluorenylformamide (10.5 g., 0.05 mole) [prepared by heating 2-fluorenamine in 98–100% formic acid, m.p. 169–170° (lit.[19] m.p. 172–

(13) From deamination of 3-chloro-9-oxo-2-fluorenamine.

(14) Prepared from 2-chloro-4-nitroaniline; m.p. 99–100°. L. M. Litvinenko, A. P. Grekov, N. N. Verkhovod, and V. P. Dzyuba, [*Zh. Obshch. Khim.*, **26**, 2524 (1956); *Chem. Abstr.*, **51**, 5008 (1957)] report m.p. 99–100°.

(15) Prepared from methyl 2-bromo-5-nitrobenzoate by reduction to the amine [which is *not distilled*, because of violent decomposition (2 mm.)] and a Schiemann reaction: b.p. 89.5–90.5° (3 mm.), $n^{25}D$ 1.5349. *Anal.* Calcd. for $C_8H_5BrFO_2$: C, 41.23; H, 2.60; F, 8.15. Found: C, 41.40; H, 2.83; F, 8.25.

(16) D. Todd, *Org. Reactions*, **4**, 384 (1948); K. Suzuki, E. K. Weisburger, and J. H. Weisburger, *J. Org. Chem.*, **26**, 2236 (1961); ref. 1a.

(17) This compound was reported previously as N-2-(1,3,4(?),7-tetrachlorofluorenyl)acetamide.[1a]

(18) This compound was reported as N-2-(7-fluoro-1,3,4(?)-trichlorofluorenyl)acetamide.[1a]

(19) E. C. Miller, T. L. Fletcher, A. Margreth, and J. A. Miller, *Cancer Res.*, **22**, 1002 (1962).

173°)] and anhydrous $FeCl_3$ (1 g.) were dissolved in acetic acid (300 ml.), with stirring, and chlorine was bubbled in at a rate of 200 ml./min. for 2 hr. at 60–70°. After 3 hr. of stirring at this temperature, the mixture was cooled to room temperature, and the product was filtered off and recrystallized from acetic acid and then from toluene giving 9.3 g. (53%), m.p. 271.5–273.5°. Another recrystallization from toluene gave an analytical sample: m.p. 273.5–274.5°; ν_{max} 3205 (amide N–H), 1667 (amide I), 818 (two adjacent hydrogens) cm.$^{-1}$.

Anal. Calcd. for $C_{14}H_7Cl_4NO$: C, 48.45; H, 2.03; Cl, 40.87; N, 4.04. Found: C, 48.69; H, 2.21; Cl, 40.78; N, 4.15.

Ethyl N-2-Fluorenylcarbamate.—2-Fluorenamine (36.2 g., 0.2 mole) was dissolved in dimethyl sulfoxide (250 ml.), and Na_2CO_3 (21.2 g., 0.2 mole) in water (100 ml.) was added. The mixture was cooled to <5° and ethyl chloroformate (24 g., 0.22 mole) was added dropwise with rapid stirring over a period of 1 hr. The reaction mixture was stirred 1 hr. more at 0–5° and a third hour at room temperature, and diluted with water. The product was recrystallized from methanol–water giving 46.7 g. (92%), m.p. 119–120.5°. Recrystallization from methanol gave an analytical sample, m.p. 120–121°.

Anal. Calcd. for $C_{16}H_{15}NO_2$: N, 5.53. Found: N, 5.46.

Ethyl N-2-(1,3,4,7-Tetrachlorofluorenyl)carbamate.—Ethyl N-2-fluorenylcarbamate (12.7 g., 0.05 mole) was chlorinated by a procedure similar to the one described for the fluorenylformamide. The product was filtered off, after dilution with water, and recrystallized from ethanol giving 12 g. (61%), m.p. 203.5–205°. After recrystallizations from ethanol, acetic acid, benzene, and finally methanol, the product melted at 193°, resolidified, and remelted at 206–208.5°; ν_{max} 3226 (amide N–H), 1700 (ester C=O), 820 (two adjacent hydrogens) cm.$^{-1}$.

Anal. Calcd. for $C_{16}H_{11}Cl_4NO_2$: C, 49.14; H, 2.84; Cl, 36.26; N, 3.58. Found: C, 49.15; H, 2.90; Cl, 36.03; N, 3.67.

3,7-Dibromo-9-oxo-2-fluorenamine.—To 7-bromo-9-oxo-2-fluorenamine[20] (2.7 g., 0.01 mole) in dimethyl sulfoxide (50 ml.) 48% HBr (1.8 ml. excess) was added.[21] The mixture was heated under reflux at 95–100° for 1 hr. and cooled. After water dilution the mixture was made alkaline with NH_4OH, and the product was isolated and recrystallized from toluene giving 2.6 g. (74%); m.p. 254–255°; ν_{max} 1709 (ketone C=O), 818 (two adjacent hydrogens) cm.$^{-1}$.

Anal. Calcd. for $C_{13}H_7Br_2NO$: C, 44.23; H, 2.00; N, 3.97. Found: C, 44.46; H, 2.13; N, 4.14.

3,7-Dibromo-2-fluorenamine.—3,7-Dibromo-9-oxo-2-fluorenamine (0.9 g.) was refluxed for 6.5 hr. in a mixture of 2,2'-oxydiethanol (60 ml.) and 85% hydrazine hydrate (10 ml.)[16] and diluted with water. The product, 0.7 g. (88%), m.p. 144.5–146.5°, was recrystallized from ethanol–water giving an analytical sample, m.p. 146.5–147.5°.

Anal. Calcd. for $C_{13}H_9Br_2N$: C, 46.05; H, 2.68. Found: C, 46.22; H, 2.97.

N-2-(3,7-Dibromofluorenyl)acetamide. A.—3,7-Dibromo-2-fluorenamine (0.2 g.) and acetic acid (15 ml.) were shaken at room temperature[22] until crystallization of the product took place. The excess acetic acid was removed by evaporation and the product, 0.2 g., was recrystallized from ethanol; m.p. 276.5–277.5°; ν_{max} 3245 (amide N–H), 1656 (amide I), 824 (two adjacent hydrogens) cm.$^{-1}$.

Anal. Calcd. for $C_{15}H_{11}Br_2NO$: Br, 41.94; N, 3.68. Found: Br, 41.84; N, 3.81.

B.—N-2-(7-Bromofluorenyl)acetamide (3 g., 0.01 mole) and N-bromosuccinimide (2 g., 0.011 mole) were dissolved in dimethyl formamide[5] (20 ml.) by warming. The solution was set aside in the dark for 2 hr. and then the precipitated product was filtered off and recrystallized from acetic acid giving 2.7 g. (71%). There was no melting point depression when the product was mixed with the compound obtained in A.

2,4-Dichloro-9-oxofluorene.—4-Chloro-9-oxo-2-fluorenamine (2 g.) was diazotized in concentrated HCl (100 ml.) at 0–5° with $NaNO_2$ (1 g.). The diazonium chloride was treated with a solution of CuCl (excess) in concentrated HCl (20 ml.). The product was chromatographed in benzene (alumina) and recrystallized from benzene–ligroin (*d* 0.68–0.70) giving 1.6 g. (73%); m.p. 152–153°; ν_{max} 1718 (ketone C=O), 731 (four adjacent hydrogens) cm.$^{-1}$.

Anal. Calcd. for $C_{13}H_6Cl_2O$: C, 62.68; H, 2.43; Cl, 28.47. Found: C, 62.53; H, 2.46; Cl, 28.33.

2,7-Diamino-1,3-dibromofluorene.—1,3-Dibromo-7-nitro-2-fluorenamine[1a] was reduced in a mixture of toluene and ethanol by Raney nickel and hydrazine hydrate[11] giving a 90% yield, m.p. 214–215° (ethanol), ν_{max} 810 (two adjacent hydrogens) cm.$^{-1}$.

Anal. Calcd. for $C_{13}H_{10}Br_2N_2$: N, 7.90. Found: N, 7.88.

N-7-(2-Amino-1,3-dibromofluorenyl)acetamide.—Acetylation of the above amine gave a 100% yield; m.p. 258.5–259° (ethanol); ν_{max} 1661 (amide I), 823 (two adjacent hydrogens) cm.$^{-1}$.

Anal. Calcd. for $C_{15}H_{12}Br_2N_2O$: Br, 40.35; N, 7.07. Found: Br, 39.93; N, 7.18, 7.12.

1,3-Dibromo-7-phenylazo-2-fluorenamine.—2,7-Diamino-1,3-dibromofluorene was condensed at room temperature with nitrosobenzene in a mixture of acetic acid and dimethyl sulfoxide. The product was chromatographed (benzene) on alumina and recrystallized from benzene–petroleum ether (b.p. 30–60°) giving a 50% yield, m.p. 214–215°.

Anal. Calcd. for $C_{19}H_{13}Br_2N_3$: C, 51.50; H, 2.96; N, 9.48. Found: C, 51.93; H, 3.03; N, 9.60.

7-Chloro-1,3-dibromo-2-fluorenamine.—7-Chloro-2-fluorenamine[20] (6.5 g., 0.03 mole) was dissolved in dimethyl sulfoxide[21] (60 ml.). To the stirred solution 48% HBr (7.9 ml.) was added. The mixture was heated at 90–100° for 1 hr., diluted with water, and made basic with NH_4OH. The product was isolated and recrystallized from toluene–ligroin giving 9.9 g. (88%), m.p. 198–199°.

Anal. Calcd. for $C_{13}H_8Br_2ClN$: Br, 42.79; N, 3.75. Found: Br, 42.51; N, 4.01.

Acknowledgment.—We wish to thank Norma Naimy and Carol-Ann Cole for assistance in the preparation of some of the starting materials and for running the infrared spectra.

(20) C. Courtot, *Ann. Chim.* (Paris), **14**, 5 (1930).

(21) T. L. Fletcher, M. J. Namkung, and H.-L. Pan, *Chem. Ind.* (London), 660 (1957).

(22) This facile, room temperature acetylation, in high yield, has been observed here previously for such a weak amine as 9-oxo-2-fluorenamine.