9/9 in 10 months, and gross tumors in all rats surviving to 12 months. The control group, the 2′,3′-Cl_2- and 3′,4-Cl_2DAB groups showed 0/10 tumors in 12 months.

2′,3 -Me_2DAB is by far the most active of the DAB derivatives so far tested and compares in activity with some of the heterocyclic analogs.[5]

Acknowledgment.—The authors are indebted to Dr. Daniel L. Weiss, Department of Pathology, University of Kentucky College of Medicine, for the microscopic evaluation of the tumors.

(5) E. V. Brown and J. J. Duffy, *J. Natl. Cancer Inst.*, **40**, 891 (1968).

Derivatives of Fluorene. XXVII. New Thiofluorenes Related to Metabolism of the Carcinogen N-2-Fluorenylacetamide. II[1a]

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An earlier publication[1b] described the synthesis of N-2-(3-methylthiofluorenyl)acetamide and related compounds for structure confirmation of a substance which was isolated by degradation of the reaction product of the carcinogen N-acetoxy-N-2-fluorenylacetamide with methionine, methionylglycine, or proteins prepared under physiological conditions.[2] In the course of this work, some new fluorenyl sulfones and thiofluorenes were prepared for the antitumor testing program of the CCNSC.

3-Mercapto-9-oxofluorene (I) was prepared by alkaline hydrolysis of the corresponding ethyl xanthate.[1b] The acetylmercapto derivative (II) was then prepared. Oxidation of 3-methylthio-9-oxofluorene[1b] with 30% H_2O_2 in AcOH gave the corresponding sulfone (III). Methyl 2-nitro-9-oxofluoren-6-yl sulfone (VI) was obtained by peroxide oxidation of methyl 2-nitro-9-oxofluoren-6-yl sulfoxide[1b] and also by vigorous nitration of 3-methylthio-9-oxofluorene. Increasingly vigorous nitrating (and oxidizing) conditions altered 3-methylthio-9-oxofluorene stepwise to the sulfoxide,[1b] nitro sulfoxide,[1b] and nitro sulfone. Reduction of the 2-nitro-9-oxofluoren-6-yl sulfone with $SnCl_2$ gave the corresponding amine (V); reduction with hydrazine hydrate in diethylene glycol gave 6-mesyl-2-aminofluorene (VI). Each of these amines was acetylated.

Antitumor activities of these compounds are shown in Table I. Compound V exhibited slightly activity; the other compounds were inactive.

TABLE I

ANTITUMOR ACTIVITY[a]

Compd	Daily dose, mg/kg	Survivors	Survival, days T/C	T/C, %
I	400	6/6	8.3/8.6	96
	400	4/4	9.5/9.4	101
	200	4/4	9.8/9.4	104
	100	4/4	10.3/9.4	109
II	400	4/4	9.5/9.4	101
	200	4/4	9.0/9.4	95
	100	4/4	10.3/9.4	109
III	400	6/6	8.8/9.2	95
	400	6/6	8.7/8.8	98
	200	6/6	9.2/8.8	104
	100	6/6	8.5/8.8	96
IV	400	4/4	8.3/9.4	88
	200	4/4	9.0/9.4	95
	100	4/4	9.3/9.4	98
V	400	6/6	9.7/9.3	104
	400	4/4	9.0/8.8	102
	200	4/4	9.0/8.8	102
	100	4/4	10.3/8.8	117
VI	400	0/4		
	200	4/4	9.8/9.4	104
	100	4/4	8.8/9.4	93
VII	400	6/6	8.8/8.6	102
	400	4/4	8.8/9.4	93
	200	4/4	9.8/9.4	104
	100	4/4	9.8/9.4	104
VIII	400	1/4	9.0/9.4	
	200	4/4	9.8/9.4	104
	100	4/4	8.8/9.4	93

[a] The screening data in this table were kindly supplied by Dr. Harry B. Wood, Jr., of the Cancer Chemotherapy National Service Center, National Institutes of Health, Bethesda, Md. Assays were performed as reported in *Cancer Chemotherapy Rept.*, **25**, 1 (1962). The tumor system used was L1210 lymphoid leukemia tested in BDF_1 mice.

Experimental Section[3]

3-Mercapto-9-oxofluorene (I).—To 3 g of ethyl 9-oxofluoren-3-yl xanthate in 30 ml of EtOH, a solution of 3 g of NaOH in 15 ml of H_2O was added and the mixture was boiled for 2 min and filtered hot. The precipitate in the acidified filtrate was filtered off and dried, giving 2 g, mp 125–130°. Two recrystallizations (C_6H_6) gave an analytical sample, mp 133–134°. *Anal.* ($C_{13}H_8OS$) C, H, S.

3-Acetylthio-9-oxofluorene (II).—Acetylation of 1 g of the foregoing compound gave 1 g, mp 147–148°. An analytical sample was prepared by recrystallizations from ligroin (*d* 0.69–0.71) and then from C_6H_6; mp 148–148.5°. *Anal.* ($C_{15}H_{10}O_2S$) C, H, S.

Methyl 9-Oxofluoren-3-yl Sulfone (III).—To a solution of 2 g of 3-methylthio-9-oxofluorene in 20 ml of glacial AcOH, 20 ml of H_2O_2 (30%) was added. The mixture was boiled for 2 min and cooled giving a yellow precipitate, mp 194–195°. An analytical sample, with unchanged melting point, was obtained by recrystallization (AcOH). *Anal.* ($C_{14}H_{10}O_3S$) C, H, S.

Methyl 2-Nitro-9-oxofluoren-6-yl Sulfone (IV). A.—Oxidation of methyl 2-nitro-9-oxofluoren-6-yl sulfoxide with H_2O_2 (30%), as above, gave a product with mp 258–259° (100%). Recrystallization ($C_6H_5CH_3$) gave an analytical sample with the same melting point. *Anal.* ($C_{14}H_9NO_5S$) N.

B.—To 25 ml of yellow fuming HNO_3 (*d* 1.49–1.50), 4 g of 3-methylthio-9-oxofluorene was added with stirring. The temperature rose to 60° and brown fumes were given off. The mixture was then heated to 75° and allowed to cool. The precipitate was filtered off, washed, and dried, giving 4.3 g, mp 225–248°. Two recrystallizations ($C_6H_5CH_3$) and one from AcOH raised the melting point to 258–259°. A mixture of this with the product in A had the same melting point.

Methyl 2-Amino-9-oxofluoren-6-yl Sulfone (V).—A mixture of 3 g of methyl 2-nitro-9-oxofluoren-6-yl sulfone, 8 g of $SnCl_2 \cdot 2H_2O$, 10 ml concentrated HCl, and 5 ml of EtOH was boiled for 10 min and worked up as usual to obtain 2.2 g of product, mp 237–241°. Recrystallizations ($C_6H_5CH_3$ and EtOH) gave mp 241–242°. *Anal.* ($C_{14}H_{11}NO_3S$) C, H, N.

N-2-(6-Mesyl-9-oxofluorenyl)acetamide (VII).—Acetylation

(1) (a) 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-CA-14,991 (T. L. F.). (b) T. L. Fletcher, M. J. Namkung, and H.-L. Pan, *J. Med. Chem.*, **10**, 936 (1967).

(2) P. D. Lotlikar, J. D. Scribner, J. A. Miller, and E. C. Miller, *Life Sci.*, **5**, 1263 (1966).

(3) Melting points were taken on a Fisher-Johns block and are corrected to standards. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Absorption bands of ir spectra were as expected; bands near 1300 and 1145 cm^{-1} were assigned to the sulfone group.

gave the amide, mp 268–270°. Recrystallization (EtOH) raised the melting point to 271–272°. *Anal.* ($C_{16}H_{13}NO_4S$) C, H, N.

6-Mesyl-2-fluorenamine (VI).—A mixture of 3 g of 6-mesyl-2-nitro-9-oxofluorene, 20 ml diethylene glycol, and 10 ml of 85% $N_2H_4 \cdot H_2O$ was heated under reflux for 2 hr and then without a condenser until the temperature of the mixture rose to 205°, after which refluxing was resumed for 2 more hr. When the mixture had cooled, it was poured into 100 ml of water to form a white precipitate, mp 181–184°. Two recrystallizaions (EtOH) gave an analytical sample, mp 184–185°. *Anal.* ($C_{14}H_{13}NO_2S$) C, H.

N-2-(6-Mesylfluorenyl)acetamide (VIII).—Acetylation of the foregoing amine, followed by crystallization (EtOH), gave an analytical sample, mp 237–238°. *Anal.* ($C_{16}H_{15}NO_3S$) N, S.

Derivatives of Fluorene. XXVIII.[1a,b] New Thiofluorenes Related to Metabolism of the Carcinogen N-2-Fluorenylacetamide. III[1c,d]

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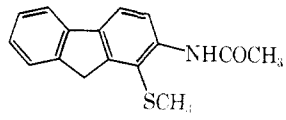
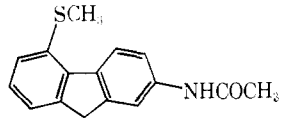
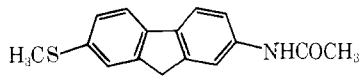
The formation of N-2-(3-methylthiofluorenyl)acetamide as a product of the *in vitro* reaction of esters of the carcinogen N-hydroxy-N-2-fluorenylacetamide with methionine under physiological conditions,[2] and the liberation of this methylthio derivative from the liver protein of rats administered the same carcinogen,[3a] led to our investigation of the syntheses of methylthiofluorene derivatives.[1c] The synthesis of the 3-methylthio derivative has been reported.[1c] This paper reports the syntheses of 1-, 5-, and 7-methylthio-2-acetamidofluorene (**1**, **2**, **3**) and their corresponding



1



2

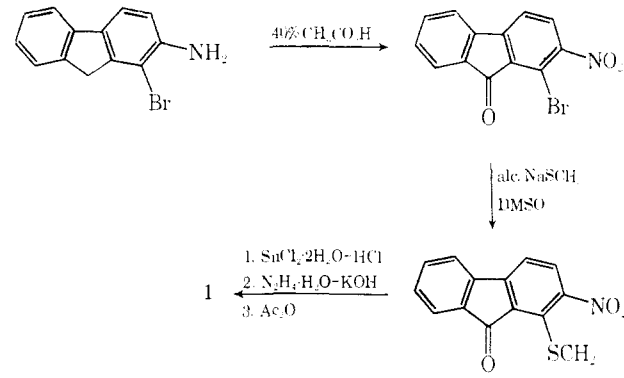


3

amines. These compounds were synthesized to facilitate the search for these derivatives as possible additional products of the reaction of these biologically important esters of N-hydroxy-N-2-fluorenylacetamide with methionine derivatives *in vitro* and *in vivo*. With these compounds as reference standards, DeBaun, Miller, and Miller (cited in ref 3b) have now identified N-2-(1-methylthiofluorenyl)acetamide as one of the products of the *in vitro* and *in vivo* reactions of esters of N-hydroxy-N-2-fluorenylacetamide with methionine; the 5- and 7-methylthio derivatives have not been detected in these reactions.

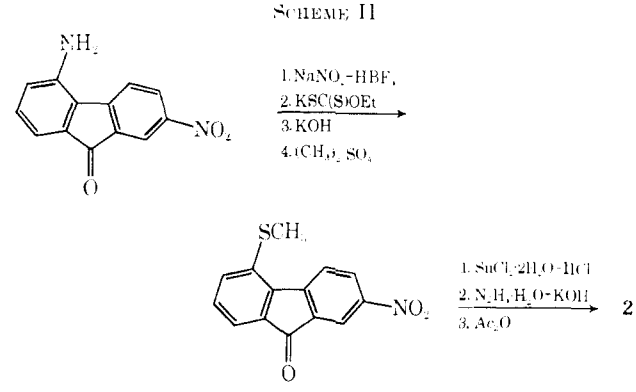
In the synthesis of **1**, 1-bromofluoren-2-amine[4] was oxidized with 40% peracetic acid to 1-bromo-2-nitro-9-oxofluorene (**4**). This was converted to the 1-methylthio derivative by treating **4** with $NaSCH_3$ in DMSO. Reduction of the 1-methylthio-2-nitro compound in two steps gave 1-methylthio-9-oxofluoren-2-amine (**5**) and 1-methylthiofluoren-2-amine (**6**). The latter was acetylated to give **1** (Scheme I).

SCHEME I



Attempts to prepare **2** and **3** by starting with 2-nitrofluoren-5-amine and 7-nitrofluoren-2-amine, respectively, were unsuccessful. However, **2** was successfully synthesized by starting with 2-nitro-9-oxofluoren-5-amine.[5] A diazonium fluoroborate was prepared from this amine and the diazonium salt was converted into a xanthate which, upon hydrolysis and methylation, gave 5-methylthio-2-nitro-9-oxofluorene. This nitro compound was first reduced to 5-methylthio-9-oxofluoren-2-amine (**7**) then to 5-methylthiofluoren-2-amine (**8**). Acetylation of the latter gave **2** (Scheme II).

SCHEME II



Preparation of **3** was accomplished in a similar manner from N-2-(7-aminofluorenyl)acetamide,[6] with the $NHCOCH_3$ group already in place. Acid hydrolysis of **3** gave us the amine **9**.

(1) (a) Paper XXV: M. J. Namkung and T. L. Fletcher, *Can. J. Chem.*, **45**, 2569 (1967). (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-CA-14,991 (T.L.F.). (c) Thiofluorenes. I: T. L. Fletcher, M. J. Namkung, and H.-L. Pan, *J. Med. Chem.*, **10**, 936 (1967). (d) Paper XXVII (Thiofluorenes. II): M. J. Namkung and T. L. Fletcher, *ibid.*, **11**, 1235 (1968).

(2) P. D. Lotlikar, J. D. Scribner, J. A. Miller, and E. C. Miller, *Life Sci.*, **5**, 1263 (1966).

(3) (a) J. R. De Baun, E. C. Miller, and J. A. Miller, *Proc. Am. Assoc. Cancer Res.*, **8**, 12 (1967); (b) J. A. Miller and E. C. Miller, *Progr. Exptl. Tumor Res.*, in press.

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

(5) F. J. Moore and E. H. Huntress, *J. Am. Chem. Soc.*, **49**, 1324 (1927).

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