o-Methoxy Derivatives of the Carcinogen, N-2-Fluorenylacetamide. Potential Latent Biological Arylating Agents[1]

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The two o-methoxy derivatives of the carcinogen, N-2-fluorenylacetamide, viz., N-(1-methoxy-2-fluorenyl)-acetamide (1b) and N-(3-methoxy-2-fluorenyl)acetamide (2b), as well as the corresponding amines, 1-methoxy-2-fluorenamine (1c) and 3-methoxy-2-fluorenamine (2c), were synthesized starting from indene and indan-1-one, and tested for carcinogenic activity. The synthetic path leading to the 3-methoxy derivative is amenable to incorporation of a ^{14}C label in the fluorene ring, heretofore not possible by other routes. Compounds 1b and 1c hydrochloride were moderately carcinogenic for the rat on feeding, but 2b and 2c hydrochloride were inactive.

A large body of experimental data supports the view that binding of chemical agents, or metabolites thereof, to proteins may play an etiological role in chemical carcinogenesis.[2] Model studies on the binding of the carcinogen, N-2-fluorenylacetamide, to proteins suggested a mechanism involving the arylation of cellular proteins by the o-fluorenoquinone imines, 2-imino-1,2-fluorenoquinone and 2-imino-2,3-fluorenoquinone. These highly reactive o-quinone imines result from the deacetylation of the *ortho*-hydroxylated metabolites of N-2-fluorenylacetamide, viz., N-(1-hydroxy-2-fluorenyl)acetamide and N-(3-hydroxy-2-fluorenyl)acetamide (2f), to the corresponding o-aminofluorenols which are then oxidized enzymatically.[3] However, the relevance of these reactions for carcinogenesis by N-2-fluorenylacetamide has remained in doubt, mainly because N-(1-hydroxy-2-fluorenyl)acetamide and the isomeric 3-hydroxy derivative (2f) were either weakly or not at all carcinogenic when administered orally, intraperitoneally or by bladder implantation.[4] While these o-amidofluorenols lacked carcinogenic activity, it is known that the carcinogenicities of many aromatic hydroxylated compounds, which include 2-amino-1-naphthol, 3-hydroxy-4-dimethylaminoazobenzene, benzo[a]pyren-3-ol, and N-(7-hydroxy-2-fluorenyl)acetamide, are greatly enhanced by methylating their phenolic hydroxyl groups.[5] The increase in biological activity may be attributable to a greater lipid solubility of the methylated derivatives which would facilitate their entry into the cell.[6] Moreover, the conjugation

to glucosiduronic acids or sulfates and the subsequent excretion of the conjugates by the kidney would be delayed until the methyl ether groups had been cleaved metabolically or until the compound had been further hydroxylated. On the basis of these considerations, the o-methoxy derivatives of N-2-fluorenylacetamide and of 2-fluorenamine were desired for carcinogenicity tests (1b, 1c, 2b, and 2c, Table I). These derivatives would not only be expected to be lipophilic, but enzymatic O-demethylation (and N-deacetylation when applicable) would release o-aminofluorenols capable of arylating cellular constituents oxidatively *via* the o-fluorenoquinon imines, i.e., these compounds could act as latent, enzyme-activated arylating agents.

The preparation of N-(1-methoxy-2-fluorenyl)acetamide (1c) for carcinogenicity tests required large quantities of fluoren-1-ol (1f) as starting material. Previously, 1f had been available from fluoranthene in six steps[7] or from indene in seven steps.[8,9] The convenient synthesis shown in Scheme I afforded the fluorenol 1f in three steps. Indenylsodium was condensed with 4-bromobutyronitrile to 4-(3-indenyl)-butyronitrile (3). This compound, described previously by Howell and Taylor,[8a] had not been completely characterized. The attachment of the butyronitrile side chain of 3 to the 3 position of indene was confirmed by its nmr spectrum which exhibited a multiplet centered at δ = 6.17 ppm due to a single
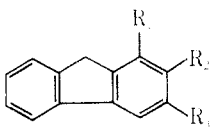
(1) Supported in part by U. S. Public Health Service Grant CA 02571.

(2) (a) E. C. Miller and J. A. Miller, *J. Natl. Cancer Inst.*, **15**, 1571 (1955); (b) C. Heidelberger in Ciba Foundation Symposium on Carcinogenesis: Mechanisms of Action, G. E. W. Wolstenholme and M. O'Connor, Ed., Little, Brown and Co., Boston, Mass., 1958, p 179; (c) D. B. Clayson, "Chemical Carcinogenesis," Little Brown and Co., Boston, Mass., 1962, pp 372–395; (d) H. C. Pitot and C. Heidelberger, *Cancer Res.*, **23**, 1694 (1963).

(3) (a) H. T. Nagasawa and H. R. Gutmann, *J. Biol. Chem.*, **234**, 1593 (1959); (b) H. R. Gutmann and H. T. Nagasawa, *ibid.*, **235**, 3466 (1960); (c) H. R. Gutmann, U. S. Seal, and C. C. Irving, *Cancer Res.*, **20**, 1072 (1960); (d) C. M. King, H. R. Gutmann, and S. F. Chang, *J. Biol. Chem.*, **238**, 2199 (1963); (e) H. T. Nagasawa and A. J. Osteraas, *Biochem. Pharmacol.*, **13**, 713 (1964).

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(5) (a) D. B. Clayson, J. W. Jull, and G. M. Bonser, *Brit. J. Cancer*, **12**, 222 (1958); (b) J. A. Miller, E. C. Miller, and G. C. Finger, *Cancer Res.*, **17**, 387 (1957); (c) J. A. Miller and E. C. Miller, *ibid.*, **21**, 1068 (1961); (d) J. W. Cook and R. Schoental, *Brit. J. Cancer*, **6**, 400 (1952); (e) G. M. Bonser, L. Bradshaw, D. B. Clayson, and J. W. Jull, ref 2b, p 215.

(6) B. B. Brodie in "Absorption and Distribution of Drugs," T. B. Binns, Ed., E. and S. Livingstone Ltd., London, 1964, pp 16–48.

(7) E. K. Weisburger and J. H. Weisburger, *J. Org. Chem.*, **18**, 864 (1953).

(8) (a) F. H. Howell and D. A. H. Taylor, *J. Chem. Soc.*, 3011 (1957); (b) M. A. Morgan and H. R. Gutmann, *J. Org. Chem.*, **24**, 1163 (1959); (c) C. C. Irving and R. F. Willard, *ibid.*, **27**, 2260 (1962).

(9) Traces of residual acid during the isolation of 3-(3-indenyl)propanol (i), an intermediate in the seven-step synthesis, catalyzes the cyclization of
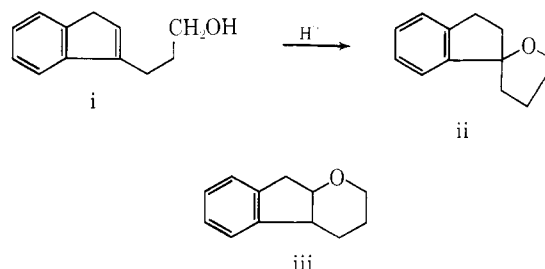

the alcohol to spiro[tetrahydrofuran-2,1'-indan] (ii), a heterocyclic system not previously described. The nmr spectrum of this product is consistent with this assignment and rules out the isomeric indenopyran structure iii.

TABLE I
o-METHOXY DERIVATIVES OF 2-SUBSTITUTED FLUORENES

No.	R_1	R_2	R_3	Yield, %	Mp, °C	Formula	Calcd, %			Found, %		
							C	H	N	C	H	N
1a	OCH₃	NO₂	H	93	100–102	$C_{14}H_{11}NO_3$	69.70	4.60	5.80	70.10	4.63	5.74
1b	OCH₃	NHCOCH₃	H	33ᵃ	146–148	$C_{16}H_{15}NO_2$	75.87	5.97	5.53	76.12	6.01	5.44
				97ᵇ	148–150							
1c	OCH₃	NH₂	H	88	107–109	$C_{14}H_{13}NO$	79.59	6.20	6.63	79.61	6.32	6.74
1d	OCH₃	N(COCH₃)₂	H	85	136–138	$C_{18}H_{17}NO_3$	73.20	5.80	4.74	73.24	5.81	4.81
2a	H	NO₂	OCH₃	33	184–185ᶜ	$C_{14}H_{11}NO_3$	69.70	4.60	5.80	69.87	4.64	5.66ᵈ
2b	H	NHCOCH₃	OCH₃	75	167–169ᵉ	$C_{16}H_{15}NO_2$	75.87	5.97	5.53	75.86	6.06	5.40ᵈ
2c	H	NH₂	OCH₃	61	164–166ᶠ	$C_{14}H_{13}NO$	79.59	6.20	6.63	79.87	6.26	6.61ᵈ

ᵃ By reductive acetylation with zinc and acetic anhydride. ᵇ By catalytic hydrogenation with Raney nickel in acetic anhydride. ᶜN. Ishikawa and M. Okazaki [*Yuki Gosei Kagaku Kyokai Shi*, **16**, 610 (1958); *Chem. Abstr.*, **53**, 3168b (1959)] report mp 183–184°. ᵈ Ishikawa and Okazakiᶜ gave independent N analyses of these compounds. ᵉ Lit.ᶜ mp 167–168°. ᶠ Lit.ᶜ mp 161–162°.
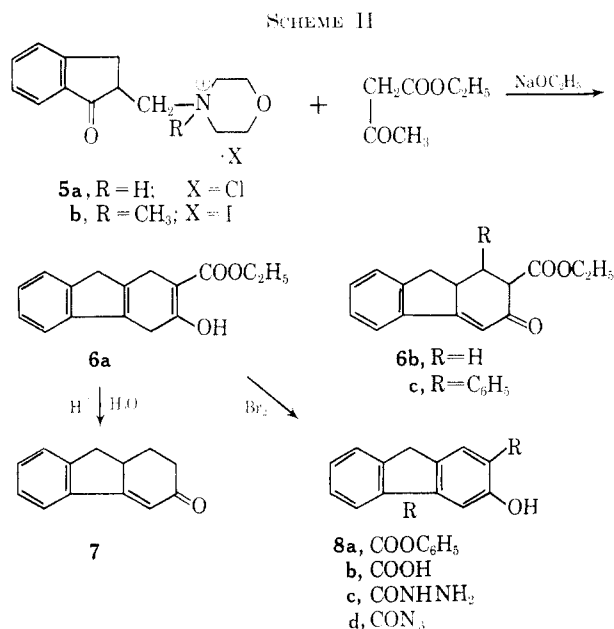
SCHEME I
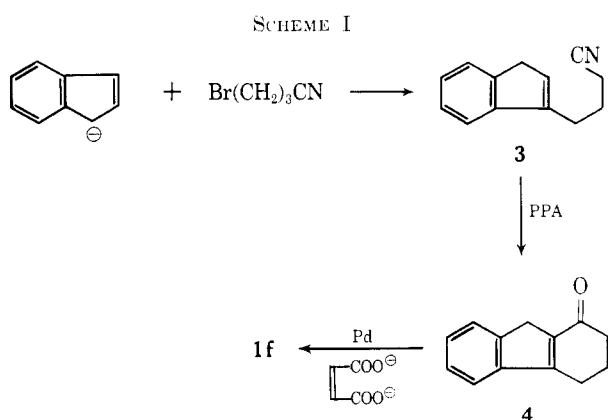
vinyl proton.[10] The nitrile **3** was then cyclized in polyphosphoric acid to 3,4-dihydrofluoren-1(2H)-one (**4**). The over-all yield of the ketone **4** from indenyl-sodium was 67%. Catalytic dehydrogenation of **4** with Pd in refluxing fluorene[8b] or catalytic transfer dehydrogenation[11] gave fluoren-1-ol (**1f**). Compound **1f** was then nitrated to 2-nitrofluoren-1-ol (**1g**)[7] and the latter was methylated to 1-methoxy-2-nitrofluorene (**1a**). 1-Methoxyfluorene when nitrated under the conditions employed for the nitration of fluoren-1-ol (**1f**) was recovered unchanged. Reductive acetylation of **1a** afforded N-(1-methoxy-2-fluorenyl)acetamide (**1b**) while catalytic hydrogenation gave 1-methoxy-2-fluorenamine (**1c**).

The isomeric N-(3-methoxy-2-fluorenyl)acetamide (**2b**) and 3-methoxy-2-fluorenamine (**2c**) were prepared in a similar manner by reductive acetylation and catalytic hydrogenation of 3-methoxy-2-nitrofluorene (**2a**).[12] The latter (**2a**) was prepared by nitration of 3-methoxyfluorene and its structure was confirmed by demethylation to the known 2-nitrofluoren-3-ol.[13] The preparation of these 3-methoxyfluorene derivatives

required fluoren-3-ol as a key intermediate. However, the chemical methods available for its preparation are not suitable for the labeling of the aromatic ring with radiocarbon for projected metabolic studies, and other approaches were investigated. The synthesis presented here provides a practical route to ¹⁴C-labeled fluoren-3-ol and therefore to ¹⁴C-labeled N-(3-hydroxy-2-fluorenyl)acetamide (**2f**) and N-(3-methoxy-2-fluorenyl)acetamide (**2b**).

Condensation of 2-(morpholinomethyl)indan-1-one hydrochloride (**5a**)[14] with ethyl sodioacetoacetate gave ethyl 1,4-dihydro-3-hydroxyfluorene-2-carboxylate (**6a**) in 73% yield[15] (Scheme II). Acid hydrolysis of **6a** gave a mixture of four components, only one of which was ketonic as shown by thin layer chromatography.

SCHEME II

5a, R = H;   X = Cl
b, R = CH₃; X = I

6a

6b, R = H
c, R = C₆H₅

7

8a, COOC₆H₅
b, COOH
c, CONHNH₂
d, CON₃

(10) The attachment of the butyronitrile side chain to the 3-position of indene is expected from the studies of A.-N. Weidler, *Acta Chem. Scand.*, **17**, 2724 (1963), who showed that in the alkylation of indenylsodium, a 1-alkylidene is first formed which rapidly rearranges to the thermodynamically more stable 3-alkylindene.

(11) L. M. Jackman in "Advances in Organic Chemistry, Methods and Results," Vol. II, R. A. Raphael, E. C. Taylor, and H. Wynberg, Ed., Interscience Publishers, Inc., New York, N. Y., 1960, pp 352–355.

(12) N. Ishikawa and M. Okazaki, *Yuki Gosei Kagaku Kyokai Shi*, **16**, 610 (1958); *Chem. Abstr.*, **53**, 3168b (1959).

(13) (a) E. K. Weisburger and J. H. Weisburger, *J. Org. Chem.*, **19**, 964 (1954); (b) K. Suzuki, E. K. Weisburger, and J. H. Weisburger, *ibid.*, **26**, 2236 (1961).
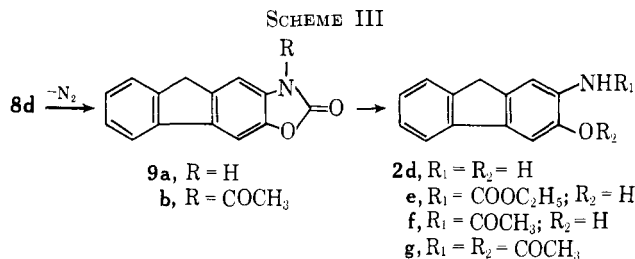
(14) R. H. Harradence and F. Lions, *J. Proc. Roy. Soc. N. S. Wales*, **72**, 284 (1938).

(15) (a) D. M. W. Anderson and D. Leaver, *J. Chem. Soc.*, 450 (1962), prepared **6a** in 31% yield by condensing the Mannich base methiodide **5b** with ethyl sodioacetoacetate and showed that the product is **6a** and not the isomeric **6b**. (b) The radiocarbon can be introduced as formaldehyde-¹⁴C in the preparation of **5a**.

After purification the ketone **7** absorbed in the infrared at 1645 cm^{-1} (C=O) indicating α,β unsaturation and, therefore, migration of the double bond during hydrolysis. The ultraviolet spectrum of **7** was similar to the reported spectra of **6b** and of its 1-phenyl analog **6c**. Its nmr spectrum (doublet centered at $\delta = 6.36$ ppm, $J = 2.5$ cps) was consistent with the presence of a vinyl proton coupled to a single allylic proton as required by structure **7**.[16] Bergmann, *et al.*,[17] have reported the preparation of a ketone, mp 96°, by the alkali-catalyzed condensation of indan-1-one with methyl vinyl ketone. Their product, presumed to be **7**, absorbed in the infrared at 1700 cm^{-1} (C=O), the position of which remained unchanged after catalytic hydrogenation of the double bond, and their 2,4-dinitrophenylhydrazone melted at 238°, while the 2,4-dinitrophenylhydrazone of **7** melted at 252–255°. It was evident, therefore, that the product obtained by Bergmann, *et al.*,[17] was not **7** but may have been the β,γ isomer instead.[18]

Catalytic dehydrogenation of **7** has been reported previously to give fluoren-3-ol in 6% yield.[14] In the present work the yield was raised to 47% by employing relatively mild conditions for the aromatization. Catalytic transfer dehydrogenation[11] with maleic anhydride as hydrogen acceptor likewise gave fluoren-3-ol in yields ranging from 45 to 59%. This method of aromatization was particularly suitable for dehydrogenations on a larger scale. The reaction was followed by thin layer chromatography and was terminated when the ketone had disappeared.

Ethyl 1,4-dihydro-3-hydroxyfluorene-2-carboxylate (**6a**) served as starting material for the preparation of a variety of fluorene derivatives and was the key intermediate for a new synthesis of N-(3-hydroxy-2-fluorenyl)acetamide (**2f**). This synthesis furnishes an alternative route to ^{14}C-labeled **2f** and, incidentally, confirms the structure of 2-aminofluoren-3-ol (**2d**)[13b] by an independent method. Bromination of **6a** gave an unstable bromine addition product which dehydrobrominated spontaneously to ethyl 3-hydroxyfluorene-2-carboxylate (**8a**). Saponification of **8a** gave the known 3-hydroxyfluorene-2-carboxylic acid (**8b**).[19] The carbethoxyfluorenol **8a** was converted to the hydrazide **8c** and then to the azide **8d** by the usual methods. The latter, when heated in toluene, rearranged with loss of nitrogen to 5H-fluoreno[2,3-*d*]-

SCHEME III

9a, R = H
b, R = COCH$_3$

2d, R$_1$ = R$_2$ = H
e, R$_1$ = COOC$_2$H$_5$; R$_2$ = H
f, R$_1$ = COCH$_3$; R$_2$ = H
g, R$_1$ = R$_2$ = COCH$_3$

oxazolin-2-one (**9a**) which on acetylation gave **9b** (Scheme III). The same fluorenooxazolinone **9a** was obtained by fusion of urea with 2-aminofluoren-3-ol (**2d**)[13] or by cyclization of its N-carbethoxy derivative **2e** in polyphosphoric acid. Hydrolysis of **9a** with 6 N HCl in a sealed tube at 160–170° gave 2-aminofluoren-3-ol (**2d**) hydrochloride which was acetylated to N-(3-hydroxy-2-fluorenyl)acetamide (**2f**) and N-(3-acetoxy-2-fluorenyl)acetamide (**2g**). Compounds **2f** and **2g** were identical in melting point, mixture melting point, and infrared spectra with samples prepared by published methods.[3b,13a,20]

Carcinogenicity Tests.—N-(1-Methoxy-2-fluorenyl)-acetamide (**1b**) and 1-methoxy-2-fluorenamine (**1c**) hydrochloride, incorporated into a 20% casein diet at a level of 0.33 g/kg of diet, when fed to the male Holtzman albino rat for 6 months, gave a tumor incidence of 27% (4/15 rats) and 57% (8/14 rats), respectively. A relatively large number of the tumors produced by **1b** and **1c** were located in the intestinal tract (2/5 and 4/9, respectively). N-(3-Methoxy-2-fluorenyl)acetamide (**2b**) and 3-methoxy-2-fluorenamine (**2c**) hydrochloride, when administered to the male Holtzman rat in the same concentration and for the same length of time as **1b** and **1c**, were not carcinogenic.

The moderate carcinogenicity exhibited by **1b** and **1c** lends partial support to the thesis[5e] that the activity of a weakly active (or inactive) hydroxylated aromatic compound, *e.g.*, N-(1-hydroxy-2-fluorenyl)acetamide, may be enhanced, or the activity of an active compound, detoxified by metabolic ring hydroxylation, may be restored by masking the free hydroxyl by an O-methyl group. The lack of carcinogenicity of **2b** and **2c** which contrasts with the activity of **1b** and **1c** can presently not be explained. Whether differences in carcinogenicity of these isomeric o-methoxy derivatives of N-2-fluorenylacetamide are attributable to differences in their metabolism by the rat must await metabolic studies with the ^{14}C-labeled compounds.

Experimental Section[21]

Spiro[tetrahydrofuran-2,1'-indan] (ii).—In the preparation of 3-(3-indenyl)propanol (i) by LiAlH$_4$ reduction of ethyl 3-(3-indenyl)propionate according to Howell and Taylor,[8a] failure to

(16) (a) Hydrolysis of **6a** in aqueous glycerol has been reported to give the ketone **7**, bp 150–173° (mostly 170–173°) (2 mm), mp 100°.[14] The elemental analysis of this product gave a value for carbon which was 2% too low. However, the coincidence of the melting points of this ketone and of its 2,4-dinitrophenylhydrazone with the melting points of the ketone **7** and of its 2,4-dinitrophenylhydrazone obtained here suggests that the two ketones are identical. Isomerization of the double bond in the 1,9a-dihydrofluoren-3(2H)-one system during distillation has been reported by D. M. W. Anderson, N. Campbell, D. Leaver, and W. H. Stafford, *J. Chem. Soc.*, 3992 (1959). (b) J. Davey and B. R. T. Keene, *Chem. Ind.* (London), 849 (1965) have recently reported that **7** can be prepared in two steps from indan-1-one and 4-piperidinobutan-2-one methiodide; however, experimental details were not available while this manuscript was in preparation.

(17) E. Bergmann, R. Ikan, and H. Weiler-Feilchenfeld, *Bull. Soc. Chim. France*, 290 (1957).

(18) Our attempts to prepare **10** or fluoren-3-ol by other routes that are potentially amenable to introducing a ^{14}C label in the molecule, *viz.*, by addition of CH$_3$MgI to the enol lactone, 4,5-dihydroindeno[1,2-*b*]pyran, followed by cyclization, or by the condensation of methyl vinyl ketone with 2-bromoindan-1-one, were unsuccessful. New compounds as well as new methods for the synthesis of known intermediates developed in the course of this work are recorded in the Experimental Section.

(19) N. Ishikawa, M. Okazaki, and M. Hayashi, *Yuki Gosei Kagaku Kyokai Shi*, **16**, 34 (1958); *Chem. Abstr.*, **52**, 5349 (1958).

(20) H. Bryant and E. Sawicki, *J. Org. Chem.*, **21**, 1322 (1956).

(21) All melting points were taken on a Fisher-Johns melting point apparatus and are corrected except where indicated (above 250°). Infrared spectra were taken on a Beckman IR-4 infrared spectrophotometer and electronic spectra on a Beckman DK-2A recording spectrophotometer by Miss I. Koehler and Mr. K. Snyker. Nmr spectra were run either as pure liquids or as solutions in deuteriochloroform on a Varian A-60 spectrometer with tetramethylsilane as internal reference. Elemental analyses were performed by the staff of the Organic Microanalytical Laboratory in the Department of Chemistry. We are indebted to K. Freude and D. Goon for technical assistance, and to Drs. G. Allen and C. Ovechka for discussions of the nmr spectra.

carefully wash the ethereal extract free from acid after the reaction resulted in the formation of a low-boiling by-product isomeric with i; bp 77–80° (0.4–0.6 mm); n^{25}D 1.5460; $\lambda_{max}^{CH_3OH}$ 272, 266, 259 mμ (ϵ_{max} 1165, 1055, 670); ν_{max}^{neat} 1060 cm^{-1} (COC). The nmr spectrum was free of peaks due to vinyl protons but showed multiplets due to aromatic protons near δ = 7.07 and a series of multiplets centered at 3.77, 2.66, and approximately 2.0 ppm in the ratio 4:2:2:6.

Anal. Calcd for $C_{12}H_{14}O$: C, 82.72; H, 8.10. Found: C, 82.64; H, 8.43.

4-(3-Indenyl)butyronitrile (3).—Sodium hydride (52.6% dispersion in mineral oil, 13.8 g, 0.304 mole) was added with stirring under a nitrogen atmosphere to 200 g (1.73 moles) of freshly distilled indene. The mixture was heated slowly to 125° at which temperature the reaction became violent and required moderation by rapid cooling in an ice bath. (It is essential that an ice bath be kept handy and the heating mantle be replaced rapidly.) A light brown solid precipitated at this point. The mixture was heated to boiling, kept under reflux for 15 min, and was then cooled to room temperature. 4-Bromobutyronitrile (56.1 g, 0.378 mole) was added rapidly through the condenser by means of a dropping funnel with vigorous agitation of the mixture. An exothermic reaction ensued which was moderated by external cooling of the flask. After 2.5 hr of stirring at room temperature (during which time the brown solid slowly dissolved and NaBr precipitated), the mixture was acidified with concentrated HCl and extracted with benzene, the benzene extract was dried (Na_2SO_4), and the solvent was removed. The residual oil was distilled collecting the product boiling at 118–137° (0.2–0.3 mm) (46.1 g, 83%). This product was contaminated with mineral oil but was suitable for use in the cyclization reaction described below. In fact, for cyclization to 4, distillation of the crude product could be omitted and the residue remaining after removal of the indene could be used directly.

To remove the mineral oil, the above distillate dissolved in hexane was percolated through an acid-washed alumina column with hexane as eluent. The early fractions containing mineral oil were discarded and the product from the latter fractions was distilled, bp 125–126° (0.1 mm) [lit.[8a] 125° (0.1 mm)]; n^{26}D 1.5616 (the product prepared according to ref 8a had n^{22}D 1.5611–1.5614); λ_{max}^{EtOH} 222, 250 mμ (ϵ_{max} 7570, 7890); ν_{max}^{neat} 2250 cm^{-1} (C≡N); δ = 6.17 ppm (multiplet, 1 proton).

Anal. Calcd for $C_{13}H_{13}N$: C, 85.21; H, 7.15; N, 7.64. Found: C, 85.14; H, 7.04; N, 7.74.

3,4-Dihydrofluoren-1(2H)-one (4).—To 325 g of polyphosphoric acid was added at room temperature 16.6 g (0.091 mole) of 3 and the viscous mixture was blended with a heavy glass rod when it turned red-violet. The flask was immersed in an oil bath and the bath was gradually heated to 120–130°. After 30 min at this temperature with occasional stirring, the reaction mixture was decanted into 1300 ml of water and stirred manually until all the acid had dissolved. The solution was then heated under reflux and stirred for 30 min during which time a dark brown oil separated. The product was taken up in $CHCl_3$, and the $CHCl_3$ extract was washed twice with water, dried (Na_2SO_4), and evaporated *in vacuo* to give a brown solid which was taken up in benzene and charged on a 2.5 × 35 cm column of acid-washed alumina (Merck). Elution with benzene–petroleum ether (bp 30–60°) (4:1) gave 4 as a light yellow solid: 13.6 g (81.5%); mp 106–107° (lit.[8a] 104–106°); $\lambda_{max}^{95\%\ EtOH}$ 232, 238, 302 mμ (ϵ_{max} 6300, 8500, 20,500); $\nu_{max}^{CCl_4}$ 1663 cm^{-1} (C=O).

Compound 4 was converted to its **oxime** in 80% yield with hydroxylamine hydrochloride in ethanolic pyridine; it was recrystallized from ethanol; mp 178–180°; $\lambda_{max}^{95\%\ EtOH}$ 237, 244, 299 mμ (ϵ_{max} 11,600, 8940, 23,800).

Anal. Calcd for $C_{13}H_{13}NO$: C, 78.40; H, 6.58; N, 7.03. Found: C, 78.68; H, 6.83; N, 7.10.

Fluoren-1-ol (1e) by Catalytic Transfer Dehydrogenation of 4.—3,4-Dihydrofluoren-1(2H)-one (4, 3.68 g, 0.020 mole) was heated under reflux in a mixture of 100 ml of glycol methyl ether, 20 ml of water, 8.0 g of K_2CO_3, and 0.36 g of Pd black. Additional 0.36 g of Pd black was added after 24 hr and heating was continued for another 17 hr. The reaction mixture was worked up in a manner similar to fluoren-3-ol below to give 2.40 g (65%) of 1e, mp 120–122° (lit.[7] 119–120.5°).

1-Methoxy-2-nitrofluorene (1a).—2-Nitrofluoren-1-ol[7] (1.05 g, 4.6 mmoles) was converted to its sodium salt with dilute NaOH and the dried salt, suspended in purified dioxane (10 ml), was methylated with dimethyl sulfate (10 ml) for 6 hr (steam bath). The excess dimethyl sulfate was decomposed with saturated

aqueous Na_2CO_3, and the mixture was extracted with benzene. The residue which remained after solvent evaporation was extracted with hexane to give crude 1a (640 mg) after solvent evaporation. Purification by chromatography on alumina (neutral) with benzene–hexane (1:1) as eluent gave 530 mg of 1a (48%). Recrystallization from ethanol–water afforded pale yellow needles: mp 100–102°; λ_{max}^{EtOH} 314 mμ (ϵ_{max} 14,600); ν_{max}^{KBr} 1350, 1555 (NO_2), 1010 cm^{-1} (COC). In an alternative procedure, 2-nitrofluoren-1-ol (200 mg, 0.88 mmole) in 5 ml of acetone was methylated with methyl iodide (5 ml) in the presence of Ag_2O[22] (0.30 g, 0.86 mmole) at 50° for 20 hr. The crude product obtained by removing the silver salts and evaporating the solvent was purified by chromatography on alumina as above to give 1a (93%), mp 100–101°.

N-(1-Methoxy-2-fluorenyl)acetamide (1b).—Compound 1a (304 mg, 1.3 mmoles) in acetic anhydride (4 ml) and pyridine (0.4 ml) was acetylated reductively with zinc dust (2.0 g). After the initial reaction had subsided, acetic anhydride (2 ml) was added, and the mixture was boiled briefly. It was then filtered and the filtrate was diluted with water (100 ml). The product which crystallized on cooling overnight was recrystallized from ethanol–water to give 105 mg (33%) of 1b, mp 146–148°. Alternatively, 1a (262 mg, 1.1 mmoles) in acetic anhydride (10 ml) was hydrogenated at room temperature and atmospheric pressure with Raney nickel catalyst to give 270 mg (97%) of 1b, mp 148–150°. The ultraviolet and infrared spectra of the two products were identical.

The material, 1b, was chromatographed on paper (solvent, 50% ethanol) previously equilibrated with the solvent for 12 hr. The compound was located as a yellow spot (R_f 0.76) by spraying the chromatogram twice with a 20% solution of $NaNO_2$ and once with 6 N HCl. The papers were then heated in an oven at 80° or exposed to steam from a steam bath.

1-Methoxy-2-fluorenamine (1c).—Compound 1a (250 mg, 1.0 mmole) in glacial acetic acid was hydrogenated with Pd catalyst (30 mg) at room temperature and atmospheric pressure. Recrystallization (ethanol) of the crude product gave 135 mg (73%) of 1c, mp 106–108°. In a larger run, 1.0 g of 1a yielded 88% of 1c. For analysis, 1c was recrystallized once again from ethanol, mp 107–109°. When chromatographed on paper with 2.4 N HCl as a solvent the compound gave a single red spot (R_f 0.25) on spraying the chromatogram with a 1% solution of p-dimethylaminobenzaldehyde in 1 N HCl.

N-(1-Methoxy-2-fluorenyl)diacetamide (1d).—Compound 1b (250 mg, 0.99 mmole) in acetic anhydride (5 ml) was heated under reflux for 4.5 hr. The solution was poured into water (50 ml) and the mixture was brought to a boil; 1d (264 mg, 90%) precipitated on cooling, mp 132–138°. Two recrystallizations from ethanol afforded pure 1d, mp 136–138°. Acetylation of 1c by the above procedure likewise gave 1d in 85% yield. The infrared spectrum of 1d showed no N–H stretching or amide II bands.

2-(Diethylmalonylmethyl)indan-1-one. A. From the Mannich Base Methiodide 5b.—To sodiomalonic ester prepared from 80.1 g (0.50 mole) of diethyl malonate and 4.83 g (0.21 g-atom) of sodium in 350 ml of absolute ethanol was added at room temperature with stirring 74.6 g (0.20 mole) of pulverized **2-(morpholinomethyl)indan-1-one methiodide (5b)** in small portions over 0.5 hr. (The crude methiodide[11] can be recrystallized, but only in small portions (< 10 g) from methanol containing some methyl iodide, mp 167–170°. *Anal.* Calcd for $C_{15}H_{20}INO_2$: C, 48.27; H, 5.40; N, 3.75. Found: C, 48.24; H, 5.46; N, 3.65.) After an additional 0.5 hr, the reaction mixture was heated under reflux for 1 hr, cooled, and concentrated *in vacuo* to approximately one-third of its original volume. The oil which separated on dilution with water (600 ml) and neutralization with concentrated HCl was extracted twice with ether (300 ml), and the ether was washed with water, dried (Na_2SO_4), and evaporated. The residual oil was distilled, the excess diethyl malonate boiling at 43–46° (0.1 mm) was removed, and the product boiling at 176–179° (0.2–0.3 mm) was collected (47.0 g, 78%). For analysis, a sample was redistilled; bp 190–192° (0.9 mm), pale yellow oil; n^{28}D 1.5135; ν_{max}^{neat} 1718, 1735 cm^{-1} (C=O).

Anal. Calcd for $C_{17}H_{20}O_5$: C, 67.09; H, 6.62. Found: C, 67.01; H, 6.63.

B. From the Mannich Base Hydrochloride 5a. The procedure was similar to A above except that 5a replaced 5b and

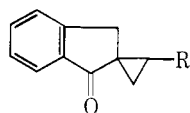(22) R. Willstätter and A. Pfannenstiel, *Chem. Ber.*, **37**, 1744 (1904).

the reaction was carried out at room temperature overnight. From sodiomalonic ester prepared from 48.1 g (0.30 mole) of diethyl malonate and 2.42 g (0.105 g-atom) of sodium and 26.8 g (0.10 mole) of **5a**, there was obtained 24.5 g (81%) of product boiling at 173–175° (0.2 mm).

Indan-1-one-2-propionic Acid.—2-(Diethylmalonylmethyl)-indan-1-one (75.8 g, 0.25 mole) was heated under reflux for 23 hr in 400 ml of 6 N H₂SO₄ which was 50% in acetic acid. The reaction mixture was then concentrated at reduced pressure to two-thirds of its original volume and poured into 1 l. of ice and water. After thorough chilling the solid product was collected, washed with cold water, and dried (45.3 g, 88%), mp 103.5–104.5°. When recrystallized from benzene–petroleum ether (bp 30–60°), the product melted at 106.5–108.5° (lit.[23] 106–108°); oxime, mp 143.5–146.5° (lit.[23a] 145°).

4,5-Dihydroindeno[1,2-*b*]pyran-2(3H)-one.—A solution of 37.1 g (0.18 mole) of indan-1-one-2-propionic acid in 400 ml of acetic anhydride was heated under reflux in a distillation apparatus for 2 hr. The acetic acid formed was then removed by distillation until the temperature of the distillate reached 130°, and the reaction mixture was further heated under reflux for 16 hr. After concentration *in vacuo* to approximately 75 ml, the remaining acetic anhydride was hydrolyzed by stirring the concentrate with ice and water for several hours, and the precipitated brownish yellow semisolid was collected. Recrystallization from ethanol gave 18.8 g (56%) of product, mp 105–106.5°.[24] For analysis, a sample was recrystallized again from ethanol, mp 106.5–107.5°, $\nu_{max}^{CCl_4}$ 1785 cm⁻¹ (C=O).

Anal. Calcd for C₁₂H₁₀O₂: C, 77.40; H, 5.41. Found: C, 77.35; H, 5.61.

Condensation of 2-Bromoindan-1-one with Methyl Vinyl Ketone.—To a stirred solution of 4.22 g (0.020 mole) of 2-bromo-indan-1-one[25] and 1.40 g (0.020 mole) of methyl vinyl ketone was added at room temperature dropwise over 10 min freshly prepared 2 N ethanolic KOH (11 ml), and the mixture was stirred at room temperature for 18 hr. The KBr which had precipitated was removed by centrifugation and washed with absolute ethanol, and the combined ethanolic solutions were evaporated to dryness. The dry residue was washed with dilute NaOH, collected, and washed with water to give 3.27 g of product melting at 103–105°. The crude material in benzene was percolated through a 1.1 × 19 cm column of alkaline alumina and eluted with benzene. The solid product which was obtained by concentrating the straw-colored effluent was recrystallized from hexane to give 2.80 g of **13a** as colorless prisms which changed crystalline modification at 95° and melted at 109–110°. An analytical sample recrystallized from benzene–petroleum ether had the same melting point; λ_{max}^{EtOH} 249, 288, 295 mμ (ε_max 16,100, 2810, 2880); $\nu_{max}^{CS_2}$ 1703, shoulder 1715 cm⁻¹ (C=O); $\nu_{max}^{CH_2Cl_2}$ 1700, shoulder 1715 cm⁻¹ (C=O); ν_{max}^{KBr} 1695 cm⁻¹ (C=O). The nmr spectrum indicated the absence of vinyl protons, and in addition to the aromatic proton multiplets showed a sharp singlet at δ = 3.20, a triplet centered at 2.78 (J = 7.5 cps), a singlet at 2.29 (CH₃), and a doublet centered at 1.70 ppm (J = 3.5 cps) in a ratio of 4:2:1:3:2. The product is therefore the spirodiketone **10a**, analogous to the spiroketonitrile **10b** formed in the condensation of 2-bromoindan-1-one with acrylonitrile.[26]

10a, R = COCH₃
b, R = CN

Anal. Calcd for C₁₃H₁₂O₂: C, 77.98; H, 6.04. Found: C, 77.83; H, 6.00.

Ethyl 1,4-Dihydro-3-hydroxyfluorene-2-carboxylate (6a).—To a vigorously stirred ethanolic solution of ethyl sodioaceto-

acetate, prepared from 117.1 g (0.90 mole) of ethyl acetoacetate, 250 ml of absolute ethanol, and 9.20 g (0.40 g-atom) of sodium, was added at room temperature solid 2-(morpholinomethyl)-indan-1-one hydrochloride (**5a**) (80.33 g, 0.30 mole) in small portions over a period of 45 min. An additional 100 ml of absolute ethanol was then added and the reaction mixture was heated under reflux for 3 hr. The solid product which precipitated on cooling was collected, washed successively with absolute ethanol, water, and ethanol, and dried at 105° to give 62.3 g of crude **6a**. This product was digested with two 500-ml portions of hot benzene and the insoluble material was removed by filtration. Concentration of the combined benzene extracts to 500 ml and dilution with 4 vol of absolute ethanol gave 52.6 g of **6a**: mp 157.5–158.5° (lit.[14,15a] 157°, 156–157°); $\nu_{max}^{CHCl_3}$ 1665 cm⁻¹ (C=O); ν_{max}^{KBr} 1663 cm⁻¹ (C=O). The ultraviolet spectrum in ethanol was identical with the reported spectrum. A second crop, 3.8 g (yield 56.4 g, 73%), mp 156.5–157.5°, was obtained by concentrating the above mother liquor to dryness and recrystallizing the residue from benzene–ethanol.

1,9a-Dihydrofluoren-3(2H)-one (7).—A suspension of 12.71 g (0.050 mole) of **6a** in ethanol (100 ml) and concentrated HCl (50 ml) was heated under reflux for 5 hr. The bright red solution was concentrated to one-half of its volume *in vacuo* and then diluted with an equal volume of water. The oil which separated and solidified was taken up in ethyl acetate, and the ethyl acetate was washed successively with 5% aqueous NaHCO₃ and water, dried (Na₂SO₄), and evaporated *in vacuo* to give 9.11 g of a pale yellow solid which melted between 84–94°. Thin layer chromatography (tlc) on cellulose powder, developed with methanol–water–benzene (35:20:1), indicated that the material was contaminated with three products which displayed yellow and blue fluorescence (Hg lamp). A fluorescence-quenching spot which was chromogenic when sprayed with acidified ethanolic 2,4-dinitrophenylhydrazine appeared at R_f 0.87–0.90. Recrystallization from ethyl acetate gave 5.61 g of pale yellow needles, mp 100–101°, after washing with a mixture of ethyl acetate–hexane (1:3) and drying in air. A second crop, 1.32 g (total 6.93 g, 75%), mp 98–99°, was obtained by evaporating the mother liquor to dryness and recrystallizing the residue from ethyl acetate–hexane (1:1). In larger runs, it was advantageous to percolate an ethyl acetate solution of the crude product through a column of alumina (activity grade I, neutral) with ethyl acetate as eluent. The product from the yellow frontal band was recrystallized as above; λ_{max}^{EtOH} 227, 234, 288, 312 mμ (ε_max 4600, 6000, 17,500, 17,800); $\nu_{max}^{CHCl_3}$ 1645 cm⁻¹ (C=O); δ = 6.36 ppm (doublet, J = 2.5 cps; 1 proton).

2,4-Dinitrophenylhydrazone: mp 252–255° uncor (recrystallized from xylene); $\lambda_{max}^{CHCl_3}$ 404 mμ (ε_max 32,400); ν_{max}^{KBr} (double bond region) 1617, 1590 cm⁻¹.

Anal. Calcd for C₁₃H₁₂O: C, 84.75; H, 6.57. Found: C, 84.91; H, 6.65.

Oxime, mp 138–139° (from ethanol–water).

Anal. Calcd for C₁₃H₁₃NO: C, 78.40; H, 6.58; N, 7.03. Found: C, 78.60; H, 6.67; N, 6.99.

Fluoren-3-ol. A. By Catalytic Transfer Dehydrogenation.—To 5.80 g (0.050 mole) of maleic acid, 7.59 g (0.055 mole) of K₂CO₃, 15 ml of water, and 50 ml of glycol methyl ether were added 1.84 g (0.010 mole) of **7** and 0.184 g of Pd black, and the mixture was heated under reflux with stirring for 66 hr. The reaction mixture was examined at intervals by tlc as follows. An aliquot (0.5 ml) was removed and evaporated to dryness, and the residue was partitioned between 2 ml of 5% aqueous NaHCO₃ and 1 ml of benzene. The benzene extract was chromatographed on cellulose powder plates as described above. (The ketone **7** (R_f ∼0.9) is fluorescence quenching when the chromatogram is viewed under a 2537-A lamp, while the fluorenol (R_f ∼0.8) exhibits a violet fluorescence. In addition the ketone is chromogenic when sprayed with acidified ethanolic 2,4-dinitrophenylhydrazine.) It was evident that products other than the fluorenol were also formed but these were not investigated. When the ketone had disappeared, the catalyst was separated and the reaction mixture was worked up in the same manner as the aliquot samples. The benzene extract was washed with water and extracted with two 50-ml portions of 7.5 N KOH. The alkaline extract was warmed on the steam bath to expel dissolved benzene, cooled in an ice bath, and acidified with concentrated HCl when the product precipitated. Recrystallization from ethanol–water gave 1.08 g (59%) of fluoren-3-ol, mp 136–138°. Recrystallization from benzene–petroleum ether using acid-washed alumina as decolorizing agent gave a cleaner

(23) (a) J. von Braun and G. Manz, *Ann. Chem.*, **468**, 258 (1929); (b) M. F. Ansell and D. H. Hey, *J. Chem. Soc.*, 2874 (1950).

(24) N. P. Shushirina, R. Ya. Levina, and M. Yu. Lur'e [*Vestn. Mosk. Univ. Ser. Mat. Mekhan., Astron., Fiz. i Khim.*, **12**, 173 (1957); *Chem. Abstr.*, **53**, 2176g (1959)] report mp 75–75.5° for a compound prepared under similar conditions.

(25) W. S. Johnson and W. E. Shelberg, *J. Am. Chem. Soc.*, **67**, 1745 (1945).

(26) H. O. House, V. Paragamian, R. S. Ro, and D. W. Wluka, *ibid.*, **82**, 1452 (1960).

product, mp 137–139° (lit.[27] 136–137°). When the K_2CO_3 was omitted in the dehydrogenation reaction, the yield of the fluorenol was 45%, after purification of the crude product by chromatography on acid-washed alumina with benzene as eluent and recrystallization from benzene–petroleum ether, mp 140–141°. The reaction can be scaled up threefold without difficulty.

B. By Catalytic Dehydrogenation.—Dehydrogenation of **7** (1.84 g) for 6.5 hr with 0.90 g of Pd in refluxing fluorene (7.0 g), purified by zone refining, gave 0.86 g (47%) of crude fluoren-3-ol.

3-Methoxyfluorene. A.—The fluoren-3-ol obtained by dehydrogenation of **7** was methylated in alkaline solution with dimethyl sulfate to give 3-methoxyfluorene (88%), mp 83–84° (lit.[12,28] 82–83°, 84–85°), after recrystallization from hexane or ethanol.

B. From 3-Methoxyfluoren-9-one.—Wolff–Kishner reduction of 3-methoxyfluoren-9-one[29] (8.27 g, 0.039 mole) in 60 ml of freshly distilled diethylene glycol with 20.0 g (0.34 mole) of 85% hydrazine hydrate and 40 ml of 20% KOH (w/v) in diethylene glycol gave 6.45 g (84%) of crude 3-methoxyfluorene, mp 79–83°. Recrystallization from methanol gave a product melting at 84–85°. The infrared spectra of the prodcts prepared by methods A and B were identical.

3-Methoxy-2-nitrofluorene (2a).—To a solution of 540 mg (2.74 mmoles) of 3-methoxyfluorene in glacial acetic acid (6 ml) was added dropwise with stirring a mixture of 8 N HNO_3 (0.28 ml) in glacial acetic acid (1 ml). After 1 hr the mixture was poured into water (100 ml) and the precipitate was collected, washed with water, and then dissolved in benzene. After drying (Na_2SO_4) and concentrating to 10 ml, the benzene solution was percolated through a column (1.5 × 35 cm) of acid-washed alumina. The yellow band was eluted with benzene and the product, mp 177–185°, obtained after solvent evaporation, was rechromatographed and then recrystallized from benzene to give 220 mg (33%) of **2a**, mp 184–185° (lit.[12] 183–184°). For analysis, the compound was recrystallized from 95% ethanol with no change of the melting point; ν_{max}^{KBr} 1340, 1525 (NO_2), 1020 cm^{-1} (COC). The ultraviolet spectrum in ethanol was essentially identical with the reported spectrum.[12]

Cleavage of 2a to 2-Nitrofluoren-3-ol.—Hydrolysis of **2a** (1.05 g, 4.4 mmoles) in glacial acetic acid (70 ml) and HBr (40 ml, 48.8%) gave 0.68 g (58%) of product after two recrystallizations from benzene–petroleum ether, mp 160–163° (lit.[13a] 160–161°). A mixture melting point with 2-nitrofluoren-3-ol prepared according to ref 13a was not depressed, and the infrared and ultraviolet spectra of the product were identical with those of the reference sample.

N-(3-Methoxy-2-fluorenyl)acetamide (2b).—Reductive acetylation of **2a** (264 mg, 1.0 mmole) as in the preparation of **1b** gave **2b** (75%) after recrystallization from ethanol–water; mp 167–169° (lit.[12] 167–168°); ν_{max}^{KBr} 1670 (C=O), 1540 (amide II), 1040 cm^{-1} (COC). The ultraviolet spectrum was in agreement with the reported spectrum.[12] The compound when chromatographed on paper and detected as described for **1b** had R_f 0.76.

3-Methoxy-2-fluorenamine (2c).—Compound **2a** (200 mg, 0.84 mmole) in glacial acetic acid was reduced catalytically (H_2, Pd) to give **2c** (61%), mp 164–166° (lit.[12] 161–162°) after recrystallization from ethanol–water; ν_{max}^{KBr} 3450, 3360 (NH), 1030 cm^{-1} (COC). The ultraviolet spectrum was essentially identical with that reported.[12] The compound migrated as a single component when chromatographed on paper and sprayed with 1% p-dimethylaminobenzaldehyde in 1 N HCl [R_f 0.19 (2.4 N HCl), R_f 0.77 (70% ethanol)].

Ethyl 3-Hydroxyfluorene-2-carboxylate (8a).—To a stirred suspension of 10.20 g (0.040 mole) of the enol ester **6a** in 300 ml of ether was added dropwise at room temperature 6.40 g (0.040 mole) of bromine at such a rate that the color of the bromine was discharged before the addition of the next drop. After all of the bromine had been added, stirring was continued for another 0.5 hr when a colorless solid precipitated. Evaporation of the ether (steam bath) caused the evolution of HBr. The residue, 9.80 g, mp 140.5–141.5°, was recrystallized from benzene to give 7.32 g of colorless needles melting at 141–142°. A second crop, 1.82 g (yield 9.14 g, 90%), mp 139–141°, was obtained by concentrating the mother liquor and diluting with ethanol.

For analysis, a sample was recrystallized again from benzene, mp 141–142.5°, ν_{max}^{KBr} 1680 cm^{-1} (C=O).

Anal. Calcd for $C_{16}H_{14}O_3$: C, 75.57; H, 5.55. Found: C, 75.67; H, 5.48.

3-Hydroxyfluorene-2-carboxylic Acid (8b).—The hydroxy ester **8a** (2.54 g, 0.010 mole) in 40 ml of 2 N NaOH, 40 ml of purified dioxane, and 10 ml of water was saponified by heating the mixture on the steam bath for 2 hr. Acidification with concentrated HCl, concentration *in vacuo* to remove the dioxane, and dilution with an equal volume of water, gave 2.26 g of crude product, mp 284–286° dec ($-CO_2$) (uncor) with sublimation >200°. Recrystallization from n-butyl alcohol (crystals washed with hexane) gave 1.90 g (85%) of **8b**, mp 286–288° dec ($-CO_2$) with sublimation >200° (lit.[19] 280–282°); **anilide**, mp 247–248° (lit.[30] 245–246°).

3-Hydroxyfluorene-2-carbohydrazide (8c).—A mixture of 25.43 g (0.100 mole) of **8a** and 100 ml of 85% hydrazine hydrate in 125 ml of dioxane was heated under reflux for 4 hr during which time the solid dissolved completely. The hot reaction mixture was diluted with 100 ml of water and cooled, to give 16.7 g (70%) of **8c**, mp 240–243° after washing and drying. A second crop, mp 233–235°, 4.44 g (18%), was obtained by concentrating the mother liquor; ν_{max}^{oil} 3430, 3330, 3230 (NH), 1645, 1535 cm^{-1} (amide).

Anal. Calcd for $C_{14}H_{12}N_2O_2$: C, 69.98; H, 5.04; N, 11.66. Found: C, 69.71; H, 4.85; N, 11.95.

3-Hydroxyfluorene-2-carboxazide (8d).—A stirred suspension of 2.40 g (0.010 mole) of **8c** in 40 ml of dioxane, and 60 ml of 2 N HCl was cooled in an ice bath and 0.76 g (0.011 mole) of $NaNO_2$ in 5 ml of water was added dropwise with vigorous stirring. The original solids were transformed into a thick suspension. Stirring was continued for 2 hr; the mixture was then diluted with 50 ml of water, and the product was collected, washed with water, and dried to give 2.02 g (80%) of crude **8d**. The product was partially converted to the fluorenooxazolinone **9a** during recrystallization, as evidenced by the appearance of infrared bands at 1782 and 1755 cm^{-1}, and therefore was not further purified. It lost N_2 between 140 and 160° and melted at 288–291° (the melting point of **9a**). The reaction could be scaled up tenfold without reduction in yield; ν_{max}^{oil} 3200 (weak, OH), 2160, 2130 (N_3), 1645 cm^{-1} (C=O).

Anal. Calcd for $C_{14}H_9N_3O_2$: C, 66.93; H, 3.61; N, 16.73. Found: C, 67.37; H, 3.72; N, 16.98.

5H-Fluoreno[2,3-d]oxazolin-2-one (9a). A. By Rearrangement of 8d.—Heating a suspension of 11.34 g (0.045 mole) of the azide **8d** in 150 ml of toluene under reflux for 24 hr gave 7.76 g (75%) of **9a**, mp 294–295° (uncor), sublimes >200°, after recrystallization from ethanol–water. The product could also be crystallized from dimethylformamide–water, dioxane–water, or glycol methyl ether. Its infrared and ultraviolet spectra were identical with **9a** prepared in B below.

Anal. Calcd for $C_{14}H_9NO_2$: C, 75.32; H, 4.06; N, 6.28. Found: C, 75.16; H, 4.36; N, 6.55.

B. By Fusion of 2d with Urea.—A blended mixture of 1.17 g (5.0 mmoles) of **2d·HCl** (prepared according to ref 13a) and 1.20 g (20 mmoles) of urea was heated gradually in an oil bath to 190°. Ammonia was liberated at approximately 175°. The temperature was maintained at 190° for 2 hr; the mixture was then cooled and water was added. The water-insoluble solid was collected, washed with water, and recrystallized (charcoal) from dioxane–water to give 0.88 g (79%) of **9a**, mp 289–291° (uncor). For analysis, a sample was recrystallized from ethanol; mp 290–291° (uncor), sublimes >200°; $\lambda_{max}^{95\% EtOH}$ 274, 287, 320 mμ (ϵ_{max} 14,900, 9950, 14,900); ν_{max}^{KBr} 1750 cm^{-1} (C=O); ν_{max}^{KBr} 1782, 1755 cm^{-1} (C=O) (dimorphic).

Anal. Calcd for $C_{14}H_9NO_2$: C, 75.32; H, 4.06; N, 6.28. Found: C, 75.26; H, 4.05; N, 6.31.

C. From Ethyl N-(3-Hydroxy-2-fluorenyl)carbamate (2e).—Compound **2e** (140 mg, 0.50 mmole) was heated at 170° for 15 min in 3.5 ml of polyphosphoric acid of 76% P_2O_5 content. The mixture was poured into water and heated on the steam bath for 15 min. The precipitate was collected by centrifugation and recrystallized from ethanol–water to give 55 mg (49%) of crude **9a**, mp 282–286° (uncor) with sublimation >200°. The infrared spectrum was essentially identical with that of the products obtained in A or B above.

(27) W. C. Lothrop, *J. Am. Chem. Soc.*, **61**, 2115 (1939).

(28) W. J. P. Neish, *Rec. Trav. Chim.*, **69**, 207 (1950).

(29) F. Ullmann and H. Bleier, *Chem. Ber.*, **35**, 4273 (1902).

(30) N. Ishikawa, N. Izawa, and M. Okazaki, *Seni-i Gakkaishi*, **15**, 209 (1959); *Chem. Abstr.*, **53**, 9673h (1959).

3-Acetyl-5H-fluoreno[2,3-d]oxazolin-2-one (9b).—Compound **9a** (3.00 g, 0.0134 mole) was acetylated by heating it in 30 ml of acetic anhydride for 3 hr. The crude product which had precipitated was recrystallized from dioxane–ethanol to give 3.20 g (90%) of **9b**: mp 263–265° (uncor); ν_{max}^{oil} 1805, 1725 cm^{-1} (C=O).

Anal. Calcd for $C_{16}H_{11}NO_2$: C, 72.44; H, 4.18; N, 5.28. Found: C, 72.53; H, 4.35; N, 5.54.

This product was also obtained directly from the azide (**8d**) in 72% yield by refluxing the latter in acetic anhydride (19 hr). Hydrolysis of **9b** in refluxing 6 N ethanolic HCl (19 hr) or in refluxing 1 N alcoholic KOH (1 hr) removed only the acetyl group to give **9a**.

Hydrolysis of 9a.—A suspension of 0.90 g (4.0 mmoles) of **9a** in 8.0 ml of 6 N HCl containing a few drops of n-amyl alcohol as wetting agent was heated in a micro Carius tube at 170° for 4 hr. The pressure was released carefully while cooling the tube in a Dry ice bath, and the solid product was extruded, pressed on a sintered-glass funnel with suction, and dried *in vacuo* over KOH to give 0.95 g (100%) of **2-aminofluoren-3-ol (2d)** hydrochloride, mp 237–240° dec. The free aminofluorenol was obtained in 67% yield by treating the hydrochloride with aqueous Na_2CO_3 and recrystallizing the product from ethanol–water. In other runs, the crude **2d** liberated from the hydrochloride was directly acetylated with acetic anhydride in ethyl acetate–pyridine to **N-(3-acetoxy-2-fluorenyl)acetamide (2g)** in 80% yield, mp 225–226° (lit.[3b] 233–234° uncor), or with acetic anhydride in aqueous Na_2CO_3 to **N-(3-hydroxy-2-fluorenyl)-acetamide (2f)** in 48% yield. In the latter case, work-up of the mother liquor after recrystallization and more vigorous acetylation of the residue derived therefrom afforded an additional 26% as the diacetate **2g**. The infrared spectra of **2d**, **2f**, and **2g** were identical with those of samples prepared previously by different routes.[3b,13a,20]

Ethyl N-(3-Hydroxy-2-fluorenyl)carbamate (2e).—To a cooled, stirred suspension of 2.34 g (10.0 mmoles) of 2-amino-fluoren-3-ol hydrochloride prepared according to ref 13a in 50 ml of water was added at once 2.16 g (20.0 mmoles) of ethyl chloroformate and then 50 ml of 0.6 N aqueous $NaHCO_3$ dropwise over 5 min. After 1 hr the reaction mixture was diluted with 100 ml of water, and the solid was collected and recrystallized from ethanol (yield 2.33 g, 86%); mp 192–193°; ν_{max}^{KBr} 3420, 3240 (OH, NH), 1700 cm^{-1} (C=O).

Anal. Calcd for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.16; H, 5.54; N, 5.52.

Potential Carcinolytic Agents.[1] IV. Diazoamino Mustards

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Two new diazoaminofluoro mustards have been synthesized as potential antitumor agents in order to exploit the postulated acidity of the tumor cells. During the attempted preparation of aromatic diazoamino mustards, N,N-bis(2-methanesulfonoxyethyl)-p-nitrosoaniline was synthesized and was found to be very effective against a variety of animal tumors.

Various authors[2,3a] of books concerned with the biochemistry of cancer seem to support the idea that the pH of tumor tissues is lower than that of the corresponding normal ones. This is reasonable, since it is known that tumor cells are characterized by a high rate of aerobic and anaerobic glycolysis.[3b] Lactic acid is a dead-end product of glycolysis in tumor cells and, according to Boxer and Devlin,[4] the reduction of pyruvic acid to lactic acid is probably the only "shuttle" available to tumor cells for oxidizing reduced diphospho-pyridine nucleotide (DPNH). A bottleneck in the electron transport might cause lactic acid accumulation in tumor cells,[5] especially if the rate of acid production is greater than the combined rates of (a) acid neutralization by buffers diffusing into the tumor cells from the arterial circulation, and (b) acid diffusing out of the cells into the venous blood. The accumulated lactic acid would decrease the pH of tumor cells to a certain limiting value[6] (*ca.* pH 6.0).

Tumor tissues have been shown to be *in vivo* more acidic (pH *ca.* 6.9) than most normal ones (pH *ca.* 7.4), although the evidence is at best circumstantial. The pH was measured with electrodes[7,8] or by determining the quantity of acid-insoluble sulfa drugs precipitated in various tissues.[8–10] (More recently, 5,5-dimethyl-2,4-oxazolidinedione[11] has been used.) Injection of glucose[3c] to the host increases the acidity of the tumor tissues to a pH of *ca.* 6.5. Many animal and human tumors exhibit this behavior although Reichard, *et al.*,[12] found no significant differences in the replacement and recycling of blood glucose in cancer and normal patients (see also ref 13 and 14).

Few investigators have attempted to exploit this physicobiochemical hypothesis as a means of obtaining selective inhibition of the growth of neoplastic cells.

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