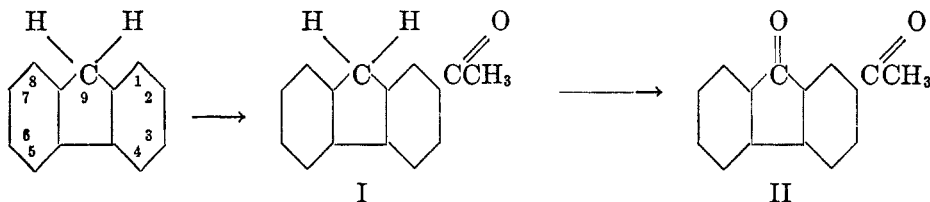


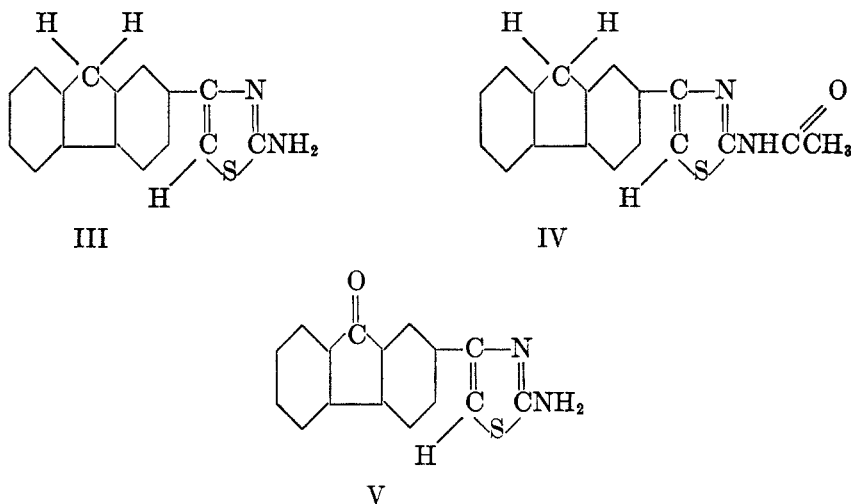
FLUORENE DERIVATIVES FOR CANCER RESEARCH¹SIDNEY SCHULMAN²*Received December 3, 1948*

During toxicity tests, Wilson, De Eds, and Cox (1) discovered that 2-acetamidofluorene and 2-aminofluorene possessed carcinogenic activity. This discovery has led to a program of synthesis to test other derivatives of the fluorene molecule for carcinogenic activity, as well as for possible cancer therapeutic action. During the work on a portion of this program a number of new fluorene compounds were prepared and a number of existing syntheses improved.

According to Dodson and King (2) ketones having two active α -hydrogens condense with thiourea in the presence of free halogen to give 4-substituted-2-aminothiazoles. Accordingly, fluorene was acetylated with acetic anhydride to yield 2-acetylfluorene, I. The latter compound was oxidized with sodium dichromate to 2-acetylfluorenone, II. The methyl ketones were condensed with thiourea in



the presence of iodine. The resulting 4-(2-fluorenyl)-2-aminothiazole and 4-(2-fluorenyl-9-one)-2-aminothiazole hydroiodide salts were isolated, and the corre-

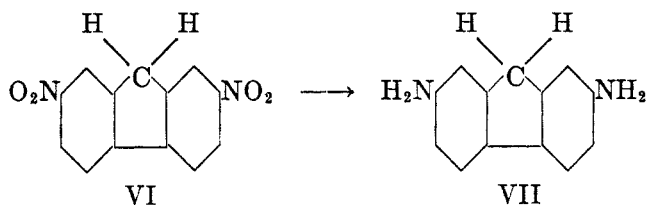


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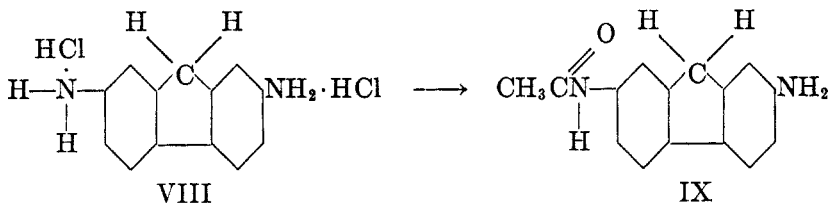
sponding free amines, III, V, then liberated by means of ammonium hydroxide. In order to characterize the amino group, 4-(2-fluorenyl)-2-aminothiazole was acetylated with acetic anhydride to yield 4-(2-fluorenyl)-2-acetamidothiazole, IV.

When 2,7-dinitrofluorene, VI, was reduced to 2,7-diaminofluorene, VII, as described by Diels (3), the isolation of the diamine from the reaction products



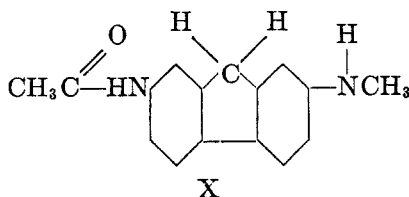
was not only cumbersome and time consuming, but gave poor yields. A simpler method of isolation of 2,7-diaminofluorene from its reaction products was devised, with yields of 90%, based on the amount of 2,7-dinitrofluorene used. A tin-2,7-diaminofluorene complex was crystallized from the partially distilled mother liquor. Dilute sulfuric acid decomposed the complex, and precipitated the sulfate of the diamine. Ammonium hydroxide liberated the free amine from its salt.

Cislak and Hamilton (4) synthesized 2-amino-7-acetamidofluorene, IX, by reducing 2-nitro-7-acetamidofluorene. However a purer product in larger yields was obtained by the addition of stoichiometric amounts of acetic anhydride to 2,7-diaminofluorene dihydrochloride, VIII. The 2-amino-7-acetamidofluorene hydrochloride was converted to the free amine with dilute ammonium hydroxide. The addition of stoichiometric amounts of 2,7-diaminofluorene and acetic anhydride in neutral anhydrous organic solvents led to mixtures of 2,7-diacetamido-

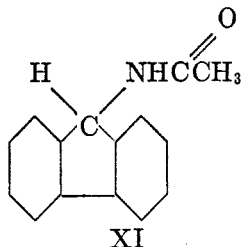


fluorene and the acetic acid salt of the diamine. Evidently the monoacetic acid salt was less basic than the monoacetylated diamine.

A new fluorene derivative, 2-N-methylamino-7-acetamidofluorene, X, was synthesized by treatment of a suspension of 2-amino-7-acetamidofluorene in benzene with dimethyl sulfate.



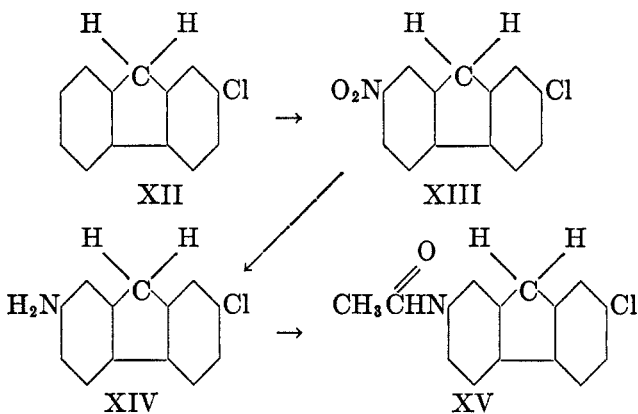
Langecker (5) reported that 9-acetamidofluorene, XI, melted at 246°, while



Schmitt (6) and Goldschmidt (7), using similar methods of production and acetic acid as the crystallization medium reported a melting point of 261°. Using a similar process, the same solvent for recrystallization and employing slow crystal growth, the author has found that 9-acetamidofluorene melted at 246°, as reported by Langecker. The apparent anomaly in melting point may be due to heteromorphic crystalline forms. This phenomenon sometimes occurs in the fluorene series when different rates of crystal formation or various solvents are used.

The synthesis of 2-nitrofluorene was greatly facilitated by modifying the experimental technique as described in Organic Syntheses (8). Using accurate temperature control rather than a water-bath, the nitrating agent was added rapidly instead of dropwise, thus saving considerable time. The exothermic reaction was also easier to control without the use of a water-bath.

2-Chloro-7-acetamidofluorene, XV, was synthesized by the acetylation of 2-chloro-7-aminofluorene, XIV, with acetic anhydride. The latter derivative was obtained by reduction of the corresponding nitro compound with zinc and calcium chloride in alcoholic solution. The direct chlorination of fluorene to produce 2-chlorofluorene, XII, followed by nitration in glacial acetic acid, yielded 2-chloro-7-nitrofluorene, XIII.



The biological testing of these compounds is being carried out at the Sloan-Kettering Institute for Cancer Research in New York City, and the findings will be published later.

I should like to thank the Sloan-Kettering Institute for Cancer Research and the University of Cincinnati for the funds allocated to this project.

EXPERIMENTAL

All melting points are uncorrected.

2-Nitrofluorene. The process recommended in Organic Syntheses (8) for the production of this compound has been modified to facilitate the temperature control and to increase the speed of production.

One liter of glacial acetic acid and 120 grams (0.72 mole) of fluorene (m.p. 107–110°) were introduced into a 2-liter 3-neck flask, equipped with a Hershberg mechanical stirrer, a thermometer, and a dropping-funnel. The suspension was stirred vigorously and heated until all the fluorene dissolved. The temperature was adjusted to 60–65°, and 160 ml. of concentrated nitric acid (sp. gr. 1.42) added in a slow steady stream. The temperature rose quickly and during this period the mixture was stirred vigorously. The temperature was not allowed to rise above 80°. A yellow precipitate separated and formed a paste. The vigorous stirring was continued to dissipate the heat, until the temperature dropped to about 60°. (When the temperature began to fall the dropping-funnel was replaced by a suction tube to draw off the oxidizing gases.) The product was filtered, sucked as dry as possible, washed with two 50-ml. portions of glacial acetic acid, each containing one gram of potassium acetate, and then with 50 ml. of glacial acetic acid. The 2-nitrofluorene was washed twice by covering with 1.5 liters of water, stirring mechanically for a few minutes, filtering, and air drying; yield 120 grams, m.p. 156–157°.

2-Acetylfluorene (I). This compound was synthesized according to the directions of Ray and Rieveschl, Jr. (9) except that double quantities were used.

2-Acetylfluorenone (II). This oxidation was carried out as described by Ray and Rieveschl, Jr. (9).

4-(2-Fluorenyl)-2-aminothiazole hydroiodide. Eighty grams (0.38 mole) of 2-acetylfluorene (m.p. 126°), sixty grams (0.86 mole) of thiourea, and 450 ml. of dioxane (peroxide free) were heated on a water-bath and 100 grams (0.40 mole) of iodine added during 2.5 hours. Heating was continued for 20 hours and after cooling an orange precipitate was filtered. The precipitate turned white upon washing with alcohol; yield 100 g. After recrystallization from 60% acetic acid the product sintered at 290° and melted to a red liquid at 306°.

4-(2-Fluorenyl)-2-aminothiazole (III). Twenty grams of 4-(2-fluorenyl)-2-aminothiazole hydroiodide was covered with 60 ml. of 10% alcoholic ammonia, the suspension mechanically stirred, filtered, and the precipitate washed with a little alcohol; m.p. 256°. The product was recrystallized from nitrobenzene (during the recrystallization an atmosphere of nitrogen was provided, otherwise oxidative impurities formed), washed with alcohol, and dried in a vacuum desiccator, or under an atmosphere of nitrogen; yield 14 g., m.p. 263°.

Anal. Calc'd for $C_{18}H_{12}N_2S$: N, 10.61. Found: N, 10.28.

4-(2-Fluorenyl)-2-acetamidothiazole (IV). Twenty grams of 4-(2-fluorenyl)-2-aminothiazole and 100 ml. (1.0 mole) of acetic anhydride were slowly brought to reflux, while being mechanically stirred. The suspension was refluxed 30 minutes, cooled, poured into 1.5 liters of cold water, and allowed to react for two hours. The suspension was filtered and the precipitate was washed with distilled water and then with acetone. The dry product sintered at 280°; m.p. 304–305°. After recrystallization from acetophenone the compound turned brown at 285°, melting at 304–305°; yield 16 g.

Anal. Calc'd for $C_{18}H_{14}N_2OS$: N, 9.15. Found: N, 8.91.

4-(2-Fluorenyl-9-one)-2-aminothiazole hydroiodide. Twenty five grams (0.11 mole) of 2-acetylfluorenone (m.p. 153–154°), 15 grams (0.23 mole) of thiourea, 130 ml. of dioxane (peroxide free), and 28 grams (0.11 mole) of iodine were heated on an oil-bath (ca. 115°) so the dioxane was in gentle reflux. The reaction mixture was cooled and filtered. The pre-

cipitate, when washed with a little alcohol, turned bright yellow; yield 25 g., sintered 195°, m.p. 303°.

4-(2-Fluorenyl-9-one)-2-aminothiazole (V). Sodium hydroxide (6 *N*) was added dropwise to an aqueous slurry of the yellow hydroiodide salt until the slurry was distinctly alkaline. The reaction mixture was filtered and the now red precipitate was dissolved in hot alcohol. The alcohol solution was filtered and 3x the volume of water was slowly added, with stirring. A red precipitate formed; sintered *ca.* 190°, m.p. 195–200°. The product was recrystallized from xylene (4 grams/100 ml. xylene); sintered 194°, m.p. 198–201°.

Anal. Calc'd for $C_{16}H_{16}N_2O$: N, 10.07. Found: N, 9.96.

2,7-Dinitrofluorene (VI). The best product was obtained by the method of Courtot (10), using yellow fuming nitric acid (sp. gr. 1.52) as the nitrating agent.

2,7-Diaminofluorene (VII). A suspension consisting of 50 g. of 2,7-dinitrofluorene (VI), 800 ml. of alcohol, 250 ml. of concentrated hydrochloric acid (sp. gr. 1.19), and 250 g. of granulated tin was refluxed until all the 2,7-dinitrofluorene had been reduced, about 2–3 hours. The reaction was filtered and 500 ml. of the solvent distilled off. The concentrated solution was allowed to cool and cream-colored tin complex salt crystals separated. The crystals were filtered, covered with 400 ml. of 3 *N* sulfuric acid, mechanically stirred, and again filtered. This precipitate was returned to a beaker, suspended in 100 ml. of water, and concentrated ammonium hydroxide added dropwise until the suspension was distinctly alkaline. Then 400 ml. of 3 *N* sulfuric acid was added slowly, the suspension stirred well, filtered, and the precipitate washed twice with 50 ml. of water. The free amine was liberated by suspending the sulfate in a mixture of 300 ml. of water and 100 ml. of concentrated ammonium hydroxide; yield 34 g., m.p. 163–164°.

2,7-Diaminofluorene dihydrochloride (VIII). 2,7-Diaminofluorene, VII, was dissolved in boiling xylene, filtered, and cooled. Dry HCl gas was passed in until no more material precipitated. The white product was filtered, washed with ether and dried, m.p. above 300°.

2-Amino-7-acetamidofluorene (IX). Thirty-five grams (0.13 mole) of 2,7-diaminofluorene dihydrochloride, VIII, was suspended in 1100 ml. of water and the suspension stirred rapidly and heated (60–70°) until all the precipitate dissolved. The clear solution was then cooled rapidly to room temperature and 13.00 ml. (0.13 mole) of acetic anhydride added dropwise, during the course of 30 minutes, with constant stirring. After all the acetic anhydride was added the mixture was stirred 60 minutes longer, then filtered, and the precipitate washed with a little water. The precipitate was placed in a beaker, covered with 400 ml. of water, and 75 ml. of concentrated ammonium hydroxide added slowly to the well-stirred suspension. The product was filtered and washed with water; sintered 125°, m.p. 140°. After three recrystallizations from alcohol the product melted at 198–199°; yield 20 g. The product was analyzed as the *hydrochloride*.

Anal. Calc'd for $C_{16}H_{15}ClN_2O$: N, 10.22. Found: N, 10.32.

The remaining 2,7-diaminofluorene was recovered by adding 10 ml. of concentrated hydrochloric acid to the mother liquor and refluxing for 20 minutes. The solution was cooled, made alkaline with ammonium hydroxide, and filtered; m.p. 162–164°.

2-N-Methylamino-7-acetamidofluorene (X). Thirty grams (0.126 mole) of 2-amino-7-acetamidofluorene, IX, was suspended in 1500 ml. of benzene and 45 grams (0.36 mole) of dimethyl sulfate added dropwise. The suspension was refluxed for ninety minutes and after cooling a light grey precipitate was filtered, m.p. above 300°. This product was stirred with ammonium hydroxide, filtered, washed with water, dried, and recrystallized from acetone. It melted to a gel *ca.* 105–110°. After one recrystallization from absolute alcohol the product sintered at 185°; m.p. 190–196°. Repeated recrystallizations from absolute alcohol gave a constant melting point of 203–204°; yield 4 g. A mixture of pure 2-amino-7-acetamidofluorene and the product sintered at 155–160°; m.p. 180–185°.

Anal. Calc'd for $C_{16}H_{16}N_2O$: N, 11.12. Found: N, 11.00.

9-Acetamidofluorene (XI). Forty grams (0.20 mole) of 9-fluorenone oxime (11) in 350 ml. of glacial acetic acid was reduced with 50 g. (0.77 mole) of zinc dust, added in small incre-

ments over a period of 30 minutes. The mixture was refluxed for an hour and after 55 ml. (0.55 mole) of acetic anhydride was added dropwise to the boiling solution it was refluxed again for an hour, filtered hot, and allowed to cool. White crystals settled out which were filtered and washed with acetic acid; yield 45 g., m.p. 246°. Subsequent recrystallization from acetic acid did not alter the melting point.

Anal. Calc'd for $C_{15}H_{13}NO$: N, 6.28. Found: N, 6.17.

2-Chlorofluorene (XII). This intermediate was synthesized according to the procedure of Courtot (10).

2-Chloro-7-nitrofluorene (XIII). The procedure was the same as that described for 2-nitrofluorene, I. The quantity of reactants used was 88 g. (0.44 mole) of 2-chlorofluorene, 810 ml. of glacial acetic acid, and 120 ml. of nitric acid (sp. gr. 1.42); yield 70 g. The crude product sintered at 210–220°; m.p. 222–235°. The product was recrystallized twice from glacial acetic acid; m.p. 243–244°. Courtot (10) found m.p. 237° (recrystallized from benzene).

2-Chloro-7-aminofluorene (XIV). Ten grams (0.04 mole) of 2-chloro-7-nitrofluorene (m.p. 243–244°) and 100 grams of zinc dust were suspended in 400 ml. of alcohol and 70 ml. of water. The suspension was refluxed for 2 hours, filtered hot, and poured into 3 liters of water; m.p. 136–138°. The product was recrystallized from 50% alcohol, with Darco; yield 7 g., m.p. 139°.

2-Chloro-7-acetamidofluorene (XV). Seven grams of 2-chloro-7-aminofluorene was dissolved in 100 ml. of hot glacial acetic acid and 20 ml. (0.20 mole) of acetic anhydride added dropwise to the refluxing solution. The solution was refluxed for 20 minutes and cooled, whereupon a white crystalline product settled out; yield 7.6 g., m.p. 228–230°. Recrystallization from glacial acetic acid gave the constant melting point 230–231°.

Anal. Calc'd for $C_{15}H_{12}ClNO$: N, 5.45. Found: N, 5.23.

SUMMARY

A number of new fluorene derivatives have been prepared and the synthesis of several other fluorene compounds has been simplified.

CINCINNATI, OHIO

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