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A New Compound for Cancer Research. Synthesis of 2-Fluoro-5-acetylaminofluorene[1]

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To investigate its carcinogenicity, 2-fluoro-5-acetylaminofluorene was synthesized from diphenic acid. In the process the following new compounds were prepared: methyl 2-nitrofluorene-5-carboxylate, methyl 2-aminofluorene-5-carboxylate, methyl 2-fluorofluorene-5-carboxylate, 2-fluorofluorene-5-carbohydrazide, 2-fluorofluorene-5-urethan, and 2-fluorofluorene-5-phthalimide.

Experimental evidence has revealed that not only the nature of the group or groups present but also their position has a considerable influence on the carcinogenicity of a compound. For example: 2-Acetylaminofluorene is a known carcinogen of fairly high potency causing a wide variety of tumors[2] while 4-acetylaminofluorene has little activity.[3] It was further shown that introduction of fluorine in the 7-position of 2-acetylaminofluorene increased its potency.[4] It is thus of interest, to determine whether the introduction of fluorine in a similar position in 4-acetylaminofluorene would similarly increase its potency.

The synthesis of the compound 2-fluoro-5-acetylaminofluorene was carried out by the following series of reactions: diphenic acid → fluorenone-4-carboxylic acid → fluorene-4-carboxylic acid → 2-nitrofluorene-5-carboxylic acid → methyl 2-nitrofluorene-5-carboxylate → methyl 2-aminofluorene-5-carboxylate → methyl 2-fluoroborodiazoniumfluorene-5-carboxylate → methyl 2-fluorofluorene-5-carboxylate → 2-fluorofluorene-5-carbohydrazide → 2-fluorofluorene-5-urethan → 2-fluorofluorene-5-phthalimide → 2-fluoro-5-aminofluorene → 2-fluoro-5-acetylaminofluorene.

Preparation of methyl 2-nitrofluorene-5-carboxylate was necessary because attempts to convert 2-aminofluorene-5-carboxylic acid directly to 2-fluoroborodiazoniumfluorene-5-carboxylic acid and subsequently to 2-fluorofluorene-5-carboxylic acid failed because of production of a large amount of tar in the pyrolysis of the diazoniumfluoroborate. Hence, the carboxyl group had to be protected. This increased the yield, although isolation of pure methyl 2-fluorofluorene-5-carboxylate still proved to be a problem which was solved by column chromatography. Next, difficulty was encountered in the acid hydrolysis of 2-fluorofluorene-5-urethan to 2-fluoro-5-aminofluorene. Conversion of the hydrazide to the isocyanate, then to the acetylated amine by refluxing with acetic anhydride[5] gave only a 20% overall yield. Hence, the modified procedure of first converting 2-fluorofluorene-5-urethan to 2-fluorofluorene-5-phthalimide was employed.

The biological activity of 2-fluoro-5-acetylaminofluorene will be reported elsewhere.

EXPERIMENTAL

Methyl 2-nitrofluorene-5-carboxylate. To 150 ml. of thionyl chloride was added in small portions 20 g. (0.078 mole) of 2-nitrofluorene-5-carboxylic acid,[6] with occasional shaking. The mixture was refluxed for 1 hr. The thionyl chloride was distilled off, then three portions of 75 ml. each of hexane were added to the flask successively and distilled again under reduced pressure to dryness. The cooled residue of acid chloride was treated with 300 ml. of dry methanol and refluxed for 17 hr. Filtration of the mixture on cooling gave a pale yellow compound, m.p. 186–187°, yield 20 g. (95%). After recrystallization from benzene brightly shining crystals, m.p. 188–189° were obtained.

Anal. Calcd. for $C_{15}H_{11}O_4N$: C, 66.91; H, 4.08; N, 5.20. Found: C, 67.26; H, 3.52; N, 5.18.

Methyl 2-aminofluorene-5-carboxylate.[7] Thirty grams (0.11 mole) of dry powdered methyl 2-nitrofluorene-5-carboxylate was made into a thin paste with 1 l. of 78% alcohol. A solution of 10 g. of calcium chloride in 15 ml. of water together with a mixture of 300 g. of zinc dust and 10 g. of Norit was added to it; the whole was thoroughly shaken and vigorously refluxed for 4 hr. At the end of the period a solution of 1 ml. of 85% hydrazine hydrate in 2 ml. of water was added; the sludge of zinc dust and charcoal was filtered from the boiling solution and extracted with 50 ml. portions of boiling 78% alcohol. The combined filtrates were then poured into 2 l. of water, whereupon a white flocculent precipitate was obtained, which was filtered and dried. Yield: 24 g. (90%), m.p. 140–141°.

A small amount of this amine was acetylated by refluxing it with acetic anhydride in ether solution. Crystallization from benzene gave white shiny plates m.p. 186°.

Anal. Calcd. for $C_{17}H_{15}O_3N$: C, 72.59; H, 5.33; N, 4.98. Found: C, 72.45; H, 5.41; N, 5.27.

Methyl 2-fluoroborodiazoniumfluorene-5-carboxylate.[8] To 240

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[1] This investigation was supported by research grant C-1066 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

[2] H. P. Morris, C. S. Dubnik, and J. M. Johnson, *J. Nat. Cancer Inst.*, **10**, 1201 (1950).

[3] H. P. Morris, C. A. Velat, B. P. Wagner, M. Dahlgard, and F. E. Ray, *J. Nat. Cancer Inst.*, in press.

[4] J. A. Miller, E. C. Miller, R. B. Sandin, and H. P. Rusch, *Cancer Research*, **12**, 283 (1952).

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

[6] Prepared from diphenic acid by the procedure of E. K. Weisburger and J. H. Weisburger, *J. Org. Chem.*, **20**, 1396 (1955).

[7] W. E. Kuhn, *Org. Syntheses*, **Coll. Vol. II**, 47 (1943).

[8] Procedure described by E. D. Bergmann, S. Berkovic, and R. Ikan, *J. Am. Chem. Soc.*, **78**, 6037 (1956).

ml. of 48% fluoroboric acid, cooled in an ice-salt bath, were added simultaneously a solution of 8 g. of sodium nitrite in a minimum quantity of water and a thick paste of 24 g. (0.1 mole) of methyl 2-aminofluorene-5-carboxylate in water in small portions, so that the mixture always contained an excess of nitrite. During the whole operation, the mixture was stirred efficiently and kept at −6°. The diazonium salt which precipitated as an emerald green mass was filtered, washed with some fluoroboric acid and then with ether, and dried; yield 36.1 g., m.p. 111–114° dec.

Methyl 2-fluorofluorene-5-carboxylate. The diazoniumfluoroborate was decomposed at 140° (bath temp.) and the product taken up with acetone. The acetone was removed by distillation; the residue was mixed with 30 g. of sodium bicarbonate and then extracted with heptane in a Soxhlet extractor. The extract was passed through a 40 × $2^1/_2$ cm. column of magnesium trisilicate.[9] The filtrate on evaporation gave crude methyl 2-fluorofluorene-5-carboxylate, m.p. 118–122°, yield 9 g. (37%, calculated on amine). A sample recrystallized several times from methanol melted at 119–120°.

Anal. Calcd. for $C_{15}H_{11}O_2F$: C, 74.38; H, 4.54; F, 7.85. Found: C, 74.25; H, 4.47; F, 8.10.

2-Fluorofluorene-5-carboxylic acid was prepared by hydrolysis of the ester with sodium hydroxide and was purified by sublimation *in vacuo*, m.p. 185–186°.

Anal. Calcd. for $C_{14}H_9O_2F$: C, 73.68; H, 3.94; F, 8.33. Found: C, 73.69; H, 3.84; F, 8.48.

2-Fluorofluorene-5-carboxamide was prepared from the acid chloride and ammonia. It was recrystallized from ethanol and formed shining needles, m.p. 215°.

Anal. Calcd. for $C_{14}H_{10}ONF$: C, 74.00; H, 4.40; N, 6.16; F, 8.37. Found: C, 73.86; H, 4.28; N, 6.11; F, 8.63.

2-Fluorofluorene-5-carbohydrazide.[10] A mixture of 10 g. (0.04 mole) of methyl 2-fluorofluorene-5-carboxylate, 10 ml. of *n*-butanol, and 19.2 ml. of 85% hydrazine hydrate was refluxed for 6 hr. under anhydrous conditions. Within 15 min. a solid appeared and increased with time. On cooling, the mixture solidified *en masse.* It was then transferred to a Büchner funnel, all the liquid was pressed out under suction, and the residue was washed with water, ether and dried; yield 8.5 g. (85%), m.p. 200–201°. Recrystallization from ethanol gave a pure white substance, m.p. 201°.

Anal. Calcd. for $C_{14}H_{11}N_2FO$: C, 69.42; H, 4.54; N, 11.57; F, 7.85. Found: C, 69.90; H, 4.82; N, 11.68; F, 7.83.

2-Fluorofluorene-5-carbonazide. The hydrazide (0.1 g.) in 10 ml. of 6*N* hydrochloric acid was treated with an aqueous solution of sodium nitrite (0.1 g.) with stirring at 0–5°. The pale yellow precipitate which formed was filtered and air-dried. It decomposed at 87°.

2-Fluorofluorene-5-urethan. A suspension of 7.3 g. (0.03 mole) of 2-fluorofluorene-5-carbohydrazide in 200 ml. of 6*N* hydrochloric acid was placed in an ice-salt bath and 400 ml. of ether was added followed by 4 g. of sodium nitrite in 15 ml. of water at a moderate rate, while being stirred rapidly. The temperature was kept between 0–5°. After 0.5 hr. the ether layer was separated and the aqueous layer extracted with 50-ml. portions of fresh ether. The combined ethereal extract was washed with water, then with 5% solution of sodium bicarbonate, then again with water, and finally dried over calcium chloride. The dry ether solution was decanted into a flask containing 250 ml. of absolute ethanol and the ether distilled until the residual volume was about 250 ml. The full heat of the steam bath was then applied and the mixture was refluxed for 1 hr. to complete the decomposition of the azide. Evaporation of ethanol under reduced pressure left 6.9 g. of urethan (yield 84.4%), m.p. 123–125°. On recrystallization from a mixture of ethanol and water the pure urethan, m.p. 132°, was obtained.

Anal. Calcd. for $C_{16}H_{14}NO_2F$: C, 70.84; H, 5.16; N, 5.16; F, 7.01. Found: C, 70.56; H, 4.97; N, 5.17; F, 7.58.

2-Fluorofluorene-5-phthalimide.[11] A mixture of 5 g. (0.018 mole) of 2-fluorofluorene-5-urethan and 10 g. of pure phthalic anhydride (freshly prepared)[12] was heated in an oil bath at 230° until the evolution of gases ceased (~45 min.). The reaction mixture was cooled to 100°, treated with small volume of alcohol and then neutralized with excess aqueous sodium bicarbonate (5%). The yellow precipitate of phthalimide thus obtained was filtered, thoroughly washed with water and dried; yield 6.3 g., m.p. 245–250°. Recrystallization from alcohol gave bright yellow crystals, m.p. 253–254°, yield 4.5 g. (75%).

Anal. Calcd. for $C_{21}H_{12}O_2NF$: C, 76.59; H, 3.64; N, 4.25; F, 5.77. Found: C, 76.53; H, 3.71; N, 4.29; F, 5.69.

2-Fluoro-5-aminofluorene hydrochloride. An alcoholic suspension of 4.5 g. (0.013 mole) of 2-fluorofluorene-5-phthalimide was warmed with 1.5 ml. (0.027 mole) of hydrazine hydrate (85%). The substance slowly dissolved and after some time a white gelatinous precipitate was thrown down. The mixture was warmed with excess of hydrochloric acid with occasional shaking and filtered. The residue was boiled with water and filtered again. The filtrate on cooling gave a thick pinkish-white fluffy precipitate of amine hydrochloride; yield 2.6 g. (80.7%) which sublimed at 250–255°.

2-Fluoro-5-acetylaminofluorene. An aqueous suspension of 2.3 g. (0.0097 mole) of 2-fluoro-5-aminofluorene hydrochloride was stirred with excess aqueous ammonium hydroxide (15%) and then allowed to stand for 15 min. The mixture was extracted with ether, using several small portions. The combined ethereal extract was dried over solid caustic soda and its volume was reduced to about 50 ml.

The solution of amine thus obtained was acetylated by refluxing it with 5.75 ml. of acetic anhydride for 1 hr. The thick white precipitate of the 2-fluoro-5-acetylaminofluorene was filtered, washed with water, and dried, yield 1.9 g. (81%), m.p. 205–207°. Repeated crystallization from ethanol gave pure creamy white needles, m.p. 207–208°.

Anal. Calcd. for $C_{15}H_{12}ONF$: C, 74.68; H, 4.97; N, 5.80; F, 7.88. Found: C, 74.63; H, 4.82; N, 5.79; F, 7.67.

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