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Synthesis of *N*-(8-Hydroxy-2-fluorenyl)acetamide

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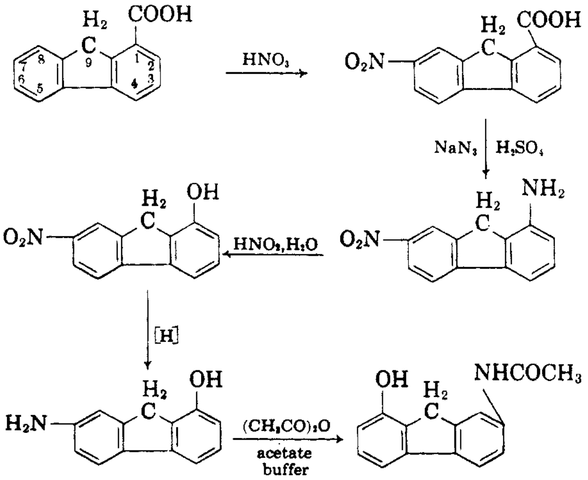
N-(8-Hydroxy-2-fluorenyl)acetamide, a minor urinary metabolite of the carcinogen *N*-2-fluorenylacetamide, was synthesized in five steps starting with 1-fluorenecarboxylic acid.

Theoretical considerations regarding the biological hydroxylation of the carcinogen N-2-fluorenylacetamide suggested that position 1, 3, 5, and 7 should be attacked, by virtue of the increased electron density of these positions contributed by the free electron pair of the nitrogen atom attached to the two position.[1] Experimental confirmation of *in vivo* hydroxylation at these centers was indeed obtained.[2]

As discussed in an earlier paper,[1] the 9 carbon atom of the fluorene molecule would tend to activate in a minor way positions 1 and 3, and for reasons of symmetry, positions 6 and 8. Thus, there is a possibility that biochemical hydroxylation at these positions might also occur, resulting in the production of N-(6- and 8-hydroxy-2-fluorenyl)-acetamide. On the other hand these compounds might also result from a non-selective dehydration of the corresponding dihydrodiols, which could be obtained as intermediates in biological hydroxylation reactions.[3]

The following method was, therefore, devised to synthesize N-(8-hydroxy-2-fluorenyl)acetamide as reference compound for our studies of the metabolism of N-2-fluorenylacetamide.

Nitration of 1-fluorenecarboxylic acid yielded the 7-nitro derivative, as proved by oxidation to the known 7-nitro-9-oxo-1-fluorenecarboxylic acid.[4] Replacement of the carboxy with the amino group by means of a Schmidt reaction gave 7-nitro-1-fluorenamine. Difficulties were encountered during the reduction of this compound to 1,7-fluorenediamine by tin and hydrochloric acid or zinc and calcium chloride in ethanol. The products gave a low analysis for carbon, even though the infrared spectra indicated the presence of a diamine. However, an excellent yield of the pure diamine was obtained by low pressure catalytic hydrogenation.



During the preparation of 7-nitro-1-fluorenol from 7-nitro-1-fluorenamine it was necessary to exclude air and to completely destroy any excess nitrite. Failure to observe these precautions resulted in the production of 7-nitro-9-oxo-1-fluorenol. Reduction of the nitrofluorenol, followed by acetylation, gave the desired N-(8-hydroxy-2-fluorenyl)-acetamide. This compound was found to be a minor metabolite of N-2-fluorenylacetamide.

EXPERIMENTAL

Melting points were uncorrected. Ultraviolet spectra were determined in 5×10^{-5} *molar* ethanol solutions on a Cary instrument. Infrared spectra were recorded on a Perkin-Elmer spectrophotometer, model 21, using the solid potassium bromide disk technique. We are indebted to Mr. P. H. Grantham for the spectra and to Dr. W. C. Alford and Mr. R. Koegel and staff for the microanalyses.

7-Nitro-1-fluorenecarboxylic acid. A solution of 40 g. of 1-fluorenecarboxylic acid[5] in 600 ml. of glacial acetic acid was cooled to 45–50°, 150 ml. of fuming yellow nitric acid was added, and the mixture slowly was heated with stirring to 70–75° when a reaction occurred and a precipitate appeared. After maintaining the mixture at this temperature for 10 minutes it was cooled. The precipitate (33 g., m.p. 284–285°, with some decomposition at 270–275°) was filtered off and washed with 50% acetic acid and water. Crystallization from 1250 ml. of glacial acetic acid gave 29.5 g. of product, m.p. 285–286°. The ultraviolet spectrum in ethanol had a maximum at 327 mμ (ϵ = 19,700) and a minimum at 267 mμ (ϵ = 3,000).

Anal. Calc'd for $C_{14}H_9NO_4$: C, 65.88; H, 3.55; N, 5.49. Found: C, 65.70; H, 3.98; N, 5.33.

Oxidation of 0.2 g. of 7-nitro-1-fluorenecarboxylic acid in 12 ml. of glacial acetic acid by refluxing with 0.8 g. of sodium dichromate in 3 ml. of 30% aqueous acetic acid for 2 hours afforded 0.125 g. of bright yellow 7-nitro-9-oxo-1-fluorenecarboxylic acid, m.p. 238–240°. The mixture m.p. with a

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(1) Weisburger and Weisburger, *J. Org. Chem.*, **20**, 1396 (1955).

(2) Weisburger, Weisburger, and Morris, *J. Natl. Cancer Inst.*, **17**, 345 (1956).

(3) Smith, *Biochem. Soc. Symposia*, No. 5, Cambridge Univ. Press, 1950, p. 15.

(4) Garascia, Fries, and Ching, *J. Org. Chem.*, **17**, 226 (1952).

(5) Bergmann and Orchin, *J. Am. Chem. Soc.*, **71**, 1111 (1949).

sample prepared by nitration of 9-oxo-1-fluorenecarboxylic acid, according to the method of Garascia, *et al.*[4] was undepressed. Both samples had identical absorption spectra.

7-Nitro-1-fluorenamine. 7-Nitro-1-fluorenecarboxylic acid (25 g.) was dissolved in 165 ml. of concentrated sulfuric acid. After the addition of 35 ml. of fuming sulfuric acid (30% SO_3) and 200 ml. of chloroform the mixture was stirred at 40–42° while 9.5 g. of sodium azide was added in small portions. Stirring was continued 3 hours longer. The acid layer was poured into ice and neutralized with a solution of 300 g. of sodium hydroxide in 1 l. of water. The yellow precipitate, 19 g., m.p. 198–201°, was extracted with xylene or benzene leaving a dark, insoluble residue. The extracts yielded 16 g. of reddish-orange material which was crystallized from ethanol (1 g./140 ml.) to give 14.5 g. of long needles, m.p. 208–209°. Further crystallization from ethanol raised the m.p. to 209–210°. The spectrum showed maxima at 228 (ϵ = 19,800) and 335 mμ (ϵ = 15,700) and minima at 221.5 (ϵ = 18,600) and 263 mμ (ϵ = 4,300) with a plateau from 285 to 292.5 mμ (ϵ = 6,900).

Anal. Calc'd for $C_{13}H_{10}N_2O_2$: C, 69.01; H, 4.46; N, 12.39. Found: C, 69.13; H, 4.55; N, 12.38.

The *acetyl derivative* which melted at 257–257.5° crystallized from acetic acid as fluffy yellow needles. The spectrum had maxima at 230 (ϵ = 18,000) and 327 mμ (ϵ = 14,610) and a minimum at 266 mμ (ϵ = 3,300).

Anal. Calc'd for $C_{15}H_{12}N_2O_3$: C, 67.15; H, 4.51; N, 10.44. Found: C, 67.42; H, 4.98; N, 10.36.

1,7-Fluorenediamine. A solution of 0.6 g. of 7-nitro-1-fluorenamine in 150 ml. of ethanol was hydrogenated in a Parr apparatus for 15 minutes at room temperature and at 50 lbs. pressure using 150 mg. of platinum oxide catalyst. The color of the solution was discharged within 2 or 3 minutes. Evaporation of the solution gave 0.58 g. of product, m.p. 160°. A sample crystallized from benzene-petroleum ether and 50% ethanol gave light tan plates melting at 160–160.5°. The ultraviolet absorption spectrum had maxima at 214 (ϵ = 43,460), 248 (ϵ = 15,620) and 287 mμ (ϵ = 26,630) and minima at 234.5 (ϵ = 13,220) and 259 mμ (ϵ = 14,820).

Anal. Calc'd for $C_{13}H_{12}N_2$: C, 79.55; H, 6.17; N, 14.28. Found: C, 79.74; H, 5.88; N, 14.13.

The *acetyl derivative* melted at 307° after crystallization from ethanol. The spectrum had maxima at 215 (ϵ = 32,340), 289 (ϵ = 24,830), and 314 mμ (ϵ = 13,820) with minima at 242 (ϵ = 9,810) and 311 mμ (ϵ = 12,710) and a shoulder at 277 mμ.

Anal. Calc'd for $C_{17}H_{16}N_2O_2$: C, 72.83; H, 5.75. Found: C, 72.69; H, 6.15.

7-Nitro-1-fluorenol. To a solution of 4.6 g. of 7-nitro-1-fluorenamine in 160 ml. of acetic acid there was added 20 ml. of water and 60 ml. of concentrated sulfuric acid. After cooling in an ice-bath 2.8 g. of sodium nitrite in 30 ml. of water was added, followed one hour later by 5 g. of urea. Stirring was continued for another hour. The diazonium solution was dropped over a period of one hour into a refluxing mixture of 200 ml. of water, 100 ml. of xylene, and 30 ml. of sulfuric acid in an atmosphere of nitrogen and refluxed one hour longer. The crude compound, 4.8 g. m.p. 234°, was crystallized from acetic acid giving 4 g. of material, m.p. 248°. Sublimation at 25 microns pressure and further crystallization from ethanol yielded bright yellow needles, m.p. 249°. The ultraviolet absorption spectrum in ethanol showed maxima at 232 (ϵ = 9,280) and 332.5 mμ (ϵ = 15,000) with minima appearing at 227 (ϵ = 9,090) and 276 mμ (ϵ = 2,270).

Anal. Calc'd for $C_{13}H_9NO_3$: C, 68.72; H, 3.99; N, 6.17. Found: C, 68.96; H, 4.26; N, 6.05.

During initial attempts to prepare this compound, the exact procedure outlined above was used except that the diazonium solution was stirred for only 15 minutes with urea and the nitrogen atmosphere was omitted. The product obtained in 15% yield, after 4 crystallizations, consisted of tan-yellow needles, m.p. 253°, which analyzed correctly for *1-hydroxy-7-nitro-9-fluorenone.* The infrared spectrum showed a strong band at 5.93 μ, corresponding to the fluorenone band, while the band at 7.15 μ usually present in fluorene derivatives was missing. The hydroxy (3.07 μ) and nitro (6.56 and 7.44 μ) bands were prominent. The ultraviolet absorption spectrum showed maxima at 240 (ϵ = 19,740), 280 (ϵ = 17,620), 321 (ϵ = 11,680), and 390 mμ (ϵ = 4,670) and minima at 226 (ϵ = 14,440), 260 (ϵ = 11,040), 303.5 (ϵ = 10,190), and 356 mμ (ϵ = 3,180).

Anal. Calc'd for $C_{13}H_7NO_4$: C, 64.73; H, 2.93; N, 5.81. Found: C, 64.73; H, 3.17; N, 5.90.

7-Amino-1-fluorenol. A solution of 2 g. of 7-nitro-1-fluorenol in 225 ml. of ethanol was hydrogenated for 9 hours at 35 lbs. pressure in a Parr apparatus, using 60 mg. of platinum oxide catalyst. There was obtained 1.7 g. of amine, charring at 221°, m.p. 224–225°. After two crystallizations from 30% ethanol the cream-colored needles darkened at 220° and melted at 225.5–226.5°. The spectrum in ethanol had maxima at 236.5 (ϵ = 10,560) and 287 mμ (ϵ = 21,910) and minima at 233 (ϵ = 10,520) and 253 mμ (ϵ = 4,380). The nitro hydroxy compound could also be reduced by the use of zinc and calcium chloride in ethanol but the yield of amine was somewhat lower.

Anal. Calc'd for $C_{13}H_{11}NO$: N, 7.10. Found: N, 6.84.

This compound gave a dye with a maximum absorption at 529 mμ when diazotized and coupled with R salt according to the test for diazotizable amine developed by Westfall and Morris.[6] Under the standard conditions of the test, an equimolar amount of 7-amino-1-fluorenol gave 92.5% of the absorption of 2-fluorenamine at 525 mμ. In the test developed for the isomeric 7-amino-2-fluorenol,[7] the 7-amino-1-fluorenol gave only 3.8% of the absorption of the standard compound at 450 mμ.

N-(8-Hydroxy-2-fluorenyl)acetamide. Acetylation of 1.2 g. of the amino hydroxy compound in acetate buffer according to the procedure used with other isomers[1] gave 1.42 g. of fine needles, m.p. 234.5–235°, after two crystallizations from 30% aqueous ethanol. The spectrum showed maxima at 288.5 (ϵ = 28,010), 297.5 (ϵ = 22,410) and 303.5 mμ (ϵ = 20,910) and minima at 252.5 (ϵ = 6,200), 296 (ϵ = 22,310) and 302 mμ (ϵ = 20,710).

Anal. Calc'd for $C_{15}H_{13}NO_2$: C, 75.29; H, 5.48. Found: C, 74.92; H, 5.59.

Carrier isotope dilution experiments performed on the urines of rats fed N-2-fluoren-9-C^{14}-ylacetamide, according to the general procedure described previously,[2] showed that 1.2–1.9% of N-(8-hydroxy-2-fluorenyl)acetamide was present as the sum of both the free and glucuronic acid conjugated compound, while 0.15% was accounted for by the free material. The corresponding values for 7-amino-1-fluorenol were 0.35% and 0.17%, respectively. The chromatographic behavior of these compounds is reported elsewhere.[8]

N-(8-Acetoxy-2-fluorenyl)diacetamide. The N-(8-hydroxy-2-fluorenyl)acetamide (56 mg.) was refluxed in 5 ml. of acetic anhydride for 1/2 hour. The solution was poured onto ice, the excess acetic anhydride neutralized with sodium bicarbonate and the precipitate formed, 59 mg., m.p. 151°, was filtered off. Two crystallizations from benzene-petroleum ether gave 22 mg. of shiny white crystals, m.p. 160°.

The spectrum showed maxima at 267 (ϵ = 25,150), 278 (ϵ = 20,360), 287.5 (ϵ = 14,770), and 299 mμ (ϵ = 12,970). Minima appeared at 237 (ϵ = 5,190), 276.5 (ϵ = 20,160), 285 (ϵ=13,770) and 295.5 mμ (ϵ = 9,180). There was a shoulder at 312 mμ (ϵ = 3,790).

Anal. Calc'd for $C_{19}H_{17}NO_4$: C, 70.57; H, 5.30; N, 4.33. Found: C, 70.74; H, 5.38; N, 4.40.

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(6) Westfall and Morris, *J. Natl. Cancer Inst.*, **8**, 17 (1947).

(7) Damron and Dyer, *J. Natl. Cancer Inst.*, **14**, 279 (1953).

(8) Weisburger, Weisburger, Morris, and Sober, *J. Natl. Cancer Inst.*, **17**, 363 (1956).