[Contribution from the Biochemistry Section, National Cancer Institute,[1] and the Laboratory of Radiochemistry, University of Cincinnati]

THE MERCURATION OF 2-AMINOFLUORENE[2]

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The preparation of the 7-iodo derivative of 2-acetylaminofluorene (AAF) was described recently (1). The biological properties of this compound are being investigated in order to determine the effect of substitution in the 7-position on the carcinogenic properties and on the detoxification (2) of AAF. At the same time it became of interest to study the influence of halogen in a different position. The synthesis of 2-acetylamino-3-iodofluorene was achieved by the mercuration of 2-aminofluorene and replacement of the organometallic substituent with iodine.

Mercuration of aromatic amines usually leads to substitution in the *para* or *ortho* position (3). Applying this to 2-aminofluorene one might expect mercuration to occur in either the 1, 3, or 7-positions with preference to the 1 or 3 positions.

The mercuration of fluorene itself has been studied but no clear cut conclusions can be reached concerning the position of the entering acetoxymercuri- group. Goswami and Das Gupta (4) reported that a mercury derivative of fluorene was formed but did not determine the position attacked. Chanussot (5) stated that mercuration did not occur in the 2-position. Miller and Bachman (6) reported obtaining 3- and 4-bromofluorenones by mercuration of fluorene followed by treatment with bromine and oxidation. Huntress, Pfister, and Pfister (7) found, however, that the 4-bromofluorenone they synthesized from fluorenone-4-carboxylic acid did not correspond with the 4-bromofluorenone reported by Miller and Bachman. On the other hand, the melting point of 3-bromofluorenone obtained by the latter authors agreed with that reported by Montagne and van Charante (8), Courtot (9), and Heilbron *et al.* (10). Hence the mercuration of the hydrocarbon seemed to give a 3-substituted fluorene.

In the present investigation it was found that refluxing aminofluorene with mercuric chloride or acetate in ethanol or acetic acid yielded black carbonaceous material and free mercury. However, when mercuric acetate and aminofluorene solutions were mixed at room temperature, the greenish salt first formed gradually changed to a 2-amino-3-acetoxymercurifluorene complex on standing overnight. This material had a definite melting point but could not be recrystallized without decomposition.

Acetylation with acetic anhydride at room temperature yielded a product which sintered and melted over a long range. However, by means of chromatography on an alumina column, the impurities were separated and 2-acetylamino-3-acetoxymercurifluorene was isolated.

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The structure of this compound was established by the following reactions. Replacement of the acetoxymercuri- group by iodine yielded an iodo-2-acetylaminofluorene different from 2-acetylamino-7-iodofluorene (1). The acetoxymercurifluorene reacted very rapidly in the cold with one mole of bromine. From this reaction 2-acetylamino-3-bromofluorene (11) was isolated. When two moles of bromine were added, the product obtained was 2-acetylamino-3,7-dibromofluorene (11, 12). This proved that the acetoxymercuri- group was in the 3-position.

The ultraviolet absorption spectra of 2-acetylamino-3- and 7-iodofluorene (Fig. 1) are decidedly different between themselves and compared with that of 2-acetylaminofluorene (13) itself. The 7-iodo substituent in the extended *para* position to the acetylamino group causes an appreciable bathochromic shift of the main peaks, as well as an enhancement of the maxima, relative to the curve of 2-acetylaminofluorene. On the other hand the spectrum of the 3-iodo derivative exhibits a slight hypsochromic shift of the main peaks while their intensities are somewhat lowered, as compared to 2-acetylaminofluorene. This can be interpreted as being due to a mutual interference of the iodo substituent with the conjugation of the acetylamino side chain.

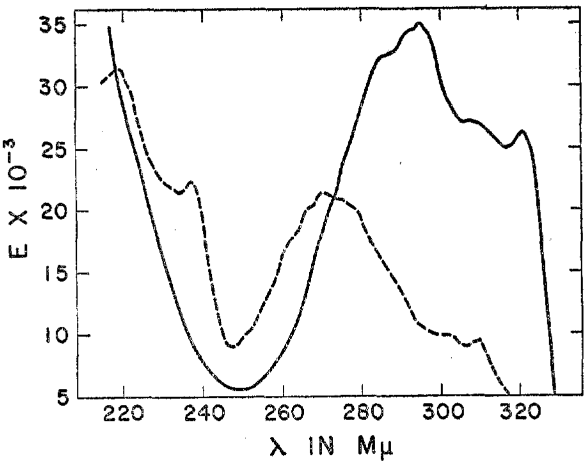

FIGURE 1. ULTRAVIOLET ABSORPTION SPECTRA: — — 2-ACETYLAMINO-3-IODOFLUORENE, ———————— 2-ACETYLAMINO-7-IODOFLUORENE

The 2-acetylamino-3-iodofluorene is being tested for carcinogenic activity by Dr. H. P. Morris of the National Cancer Institute.

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EXPERIMENTAL

2-Acetylamino-3-acetoxymercurifluorene. A solution of 45 g. of 2-aminofluorene in 1 l. of ethanol was cooled to 20°. A solution of 80 g. of mercuric acetate in 500 cc. of water, containing 10 cc. of acetic acid to prevent hydrolysis, was cooled to 15° and added slowly to the aminofluorene solution, giving a greenish precipitate; on standing overnight the color changed to brown. The next day the solid, weighing 120 g., m.p. 173–175°, was filtered off and washed. The analysis of a sample triturated with ethanol and acetone was C, 34.4; H, 3.0; Hg, 48.2; N, 2.5. This indicated that the material was probably a 2-amino-3-acetoxymercurifluorene·aminofluorene·mercuric acetate complex [calc'd for $C_{15}H_{13}HgNO_2 \cdot C_{13}H_{11}N \cdot 2(C_4H_6O_4Hg)$: C, 34.4; H, 2.9; Hg, 47.8; N, 2.2].

A mixture of 50 g. of the mercury complex, 500 cc. of glacial acetic acid, and 50 cc. of acetic anhydride was stirred mechanically for four hours, heated gently for fifteen minutes, and then poured into 4 l. of water. After standing for several hours the light brown precipitate was filtered off and washed. It weighed 33–35 g. and sintered from 95° to 145°.

For purification 5 g. was dissolved in 300 cc. of chloroform. The dark solution was passed through an activated alumina column 5 cm. in diameter and 15 cm. high. In descending order a light brown, a pinkish, and a thin yellow band formed. Elution with chloroform and ethanol removed the pinkish and yellow bands. The material from these bands appeared to be impure 2-acetylaminofluorene. The brown band was eluted by acetic acid. The acetic acid solution when diluted with water yielded 2.5 g. of brownish material. Crystallization of 1 g. from 80 cc. of chloroform afforded 0.4 g. of white microcrystalline 2-acetylamino-3-acetoxymercurifluorene, m.p. 237°.

Anal. Calc'd for $C_{17}H_{15}HgNO_2$: C, 42.3; H, 3.1; Hg, 41.6.

Found: C, 43.0; H, 3.4; Hg, 41.1.

2-Acetylamino-3-bromofluorene. A solution of 0.48 g. (0.001 mole) of 2-acetylamino-3-acetoxymercurifluorene in 75 cc. of chloroform was cooled in an ice bath. A solution of 0.055 cc. of bromine (0.001 mole) in 10 cc. of chloroform was added dropwise with stirring. The bromine color disappeared immediately. After standing for approximately 20 minutes, the solution was washed with water. Upon taking the chloroform solution to dryness a white material, m.p. 180° was obtained. This was crystallized once from acetone and three times from ethanol to give 2-acetylamino-3-bromofluorene, m.p. 205–207° (12).

2-Acetylamino-3,7-dibromofluorene. Under the same conditions 0.11 cc. (0.002 moles) of bromine in chloroform was added to the mercury derivative. After standing for one hour, the slightly yellow chloroform solution was washed with dilute bisulfite solution and water and taken to dryness. The light tan material sintered at 170° and melted at 204°. It was crystallized three times from acetone and ethanol to yield fluffy white needles of 2-acetylamino-3,7-dibromofluorene, m.p. 272–273° (11) (12).

Anal. Calc'd for $C_{15}H_{11}Br_2NO$: C, 47.3; H, 2.9; N, 3.7.

Found: C, 47.4; H, 3.1; N, 3.5.

2-Acetylamino-3-iodofluorene. A mixture of 1 g. of 2-acetylamino-3-acetoxymercurifluorene, 3 g. of potassium iodide, 0.9 g. of iodine, 40 cc. of chloroform and 50 cc. of water was heated with stirring for 15–20 minutes. The chloroform layer was washed with dilute bisulfite solution and water. Removal of the solvent gave a crude product weighing 1 g., m.p. 188–192°. Recrystallizations from benzene and ethanol yielded 0.3 g. of 2-acetylamino-3-iodofluorene in the form of fluffy white needles, m.p. 204–206°. A mixture with 2-acetylamino-7-iodofluorene (m.p. 225°) sintered at 165°, melted at 172–185°.

Anal. Calc'd for $C_{15}H_{12}INO$: I, 36.4; N, 4.0.

Found: I, 36.5; N, 4.2.

For large-scale preparations it was found that crude 2-acetylamino-3-acetoxymercurifluorene could be used, since the impurities did not interfere. From 34 g. of crude material, 16.5 g. of iodine, and 50 g. of potassium iodide in 350 cc. of chloroform and 1 l. of water, after refluxing for one hour, 28–32 g. of crude 2-acetylamino-3-iodofluorene m.p. 155–165°, was obtained. It was crystallized once from benzene and twice from ethanol using Darco, to yield 7–10 g. (in various runs) of pure material, m.p. 204–206°. The average over-all yield from 2-aminofluorene was 23%.

The ultraviolet absorption spectra of 2-acetylamino-3- and 7-iodofluorene were determined in a Beckman DU spectrophotometer. A solution of 4 mg. of each compound in 1000 ml. of 95% ethanol was used. Readings were taken at 20 A intervals, with 5 Å at the peaks. The maxima of absorption ($\epsilon_m \times 10^{-3}$) and the corresponding wave lengths (in mμ) were as follows: 2-Acetylamino-3-iodofluorene; 31.4, 219; 22.4, 237.5; 21.3, 270.5; 9.9, (300); 9.4, 310. Inflection points are cited in parentheses. 2-Acetylamino-7-iodofluorene; 32.5, (287); 40.1, 295; 27.2, 307.5; 26.3, 320.5. The minima were: 3-iodo derivative; 21.4, 234; 9.0, 248; 9.1, 306; 7-iodo derivative; 5.6, 248; 27.1, 306; 25.0, 316.

2-Amino-3-iodofluorene. A solution of 1 g. of 2-acetylamino-3-iodofluorene in 10 cc. of 10% alcoholic potassium hydroxide solution was refluxed gently for one-half hour. After addition of 40 cc. of water, the mixture was cooled and filtered. The cream colored material, weighing 0.8 g., sintered at 95° and melted gradually up to 113°. A solution of the amine in 30 cc. of benzene was passed through a column of Merck alumina (2 cm. diameter, 4 cm. high). The column was then washed with 20 cc. of benzene. The yellow eluates were evaporated to 10 cc. and 20 cc. petroleum ether added. The cream colored crystals, m.p. 114–116°, weighed 0.5 g. Six further crystallizations from benzene-petroleum ether yielded white prisms, m.p. 148–150°.

Anal. Calc'd for $C_{13}H_{10}IN$: I, 41.3. Found: I, 41.4.

An attempt was made to purify the amine by crystallization of the hydrochloride followed by conversion to the free amine. In this case the product lost iodine, so that after several crystallizations only 2-aminofluorene could be isolated. The effect may have been due to the electronegative nature of the NH_3^+ ion.

SUMMARY

The mercuration of 2-aminofluorene with mercuric acetate at room temperature has been found to occur in the 3-position. This afforded a method for the preparation of 2,3-disubstituted fluorene derivatives.

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