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8-(*N*-2-Fluorenylacetamido)guanosine, an Arylamidation Reaction Product of Guanosine and the Carcinogen *N*-Acetoxy-*N*-2-fluorenylacetamide in Neutral Solution*

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ABSTRACT: The carcinogen *N*-acetoxy-*N*-2-fluorenylacetamide and guanosine reacted readily at neutrality to yield a compound which was identified as 8-(*N*-2-fluorenylacetamido)guanosine. This compound was hydrolyzed by weak alkali at 37° to 8-(*N*-2-fluorenylamino)guanosine and by 1 N hydrochloric acid at 100° to 8-(*N*-2-fluorenylacetamido)guanine and 8-(*N*-2-fluorenylamino)guanine. The latter compound was synthesized by acid hydrolysis of the condensation product of 8-bromoguanosine 2′,3′,5′-triacetate and 2-fluorenamine.

N-Acetoxy-*N*-2-fluorenylacetamide also reacted with deoxyguanosine 5′-phosphate at neutrality to yield 8-(*N*-2-fluorenylacetamido)deoxyguanosine 5′-phosphate which was stable for 6 days at pH 7.2 and 37° and was hydrolyzed by weak acid at 37° to 8-(*N*-2-fluorenylacetamido)guanine. Guanine in soluble ribonucleic acid (sRNA) and deoxyribonucleic acid (DNA) reacted in the same fashion with *N*-acetoxy-*N*-2-fluorenylacetamide; acid hydrolysis of these nucleic acids after this reaction yielded a product identical with 8-(*N*-2-fluorenylamino)guanine.

It is generally considered that interaction of a chemical carcinogen or metabolite thereof with critical tissue constituents is necessary for carcinogenesis by these substances (reviewed in Miller and Miller, 1966). The central role of nucleic acids in replication and transfer of genetic information has thus made interactions of carcinogens with nucleic acid constituents of particular interest. Administration to rats of the general carcinogen *N*-2-fluorenylacetamide (FAA)[1] (Weisburger and Weisburger, 1958) or its more carcinogenic *N*-hydroxy metabolite (*N*-hydroxy-FAA) (Miller *et al.*, 1961) labeled with ^{14}C in the 9 position were shown by Farber and his associates (Farber *et al.*, 1962; Marroquin and Farber, 1962, 1965), Williard and Irving (1964), Miller *et al.* (1964), and Sporn and Dingman (1966) to result in the incorporation of ^{14}C into hepatic DNA and RNA. Since neither FAA nor *N*-hydroxy-FAA reacts with nucleic acids *in vitro* (Miller *et al.*, 1966), some further metabolite was implicated in the reaction. Kriek (1965, 1966), showed that *N*-hydroxy-2-fluorenamine, a probable

* From the McArdle Laboratory for Cancer Research, University of Wisconsin Medical Center, Madison, Wisconsin 53706. *Received September 19, 1966.* This work was supported by Grants CA-07175 and CRTY-5002 of the National Cancer Institute, U. S. Public Health Service, by a grant from the Jane Coffin Childs Memorial Fund for Medical Research, and by the Alexander and Margaret Stewart Trust Fund.

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[1] Abbreviation used in this paper: FAA, *N*-2-fluorenylacetamide (alternative nomenclature: AAF, 2-acetylaminofluorene).

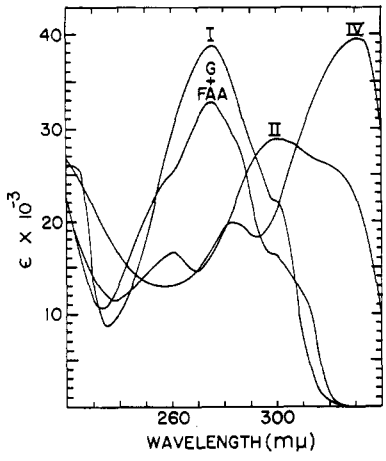

FIGURE 1: Ultraviolet absorption spectra of 8-(*N*-2-fluorenylacetamido)guanosine·0.5H_2O (I) (0.0186 mmole/l.) in 2 mM sodium citrate buffer (pH 7.0), an equimolar mixture of guanosine (G) and FAA (0.0186 mmole of each/l.) in 2 mM sodium citrate buffer (pH 7.0) containing 20% ethanol, 8-(*N*-2-fluorenylamino)-guanosine (II) (0.0170 mmole/l.) in 2 mM sodium citrate buffer (pH 7.0), and 8-(*N*-2-fluorenylamino)-guanine·H_2O·HCl (IV) (0.0051 mmole/l.) in 95% ethanol.

in vivo metabolite of FAA, and *N*-hydroxy-FAA (Irving, 1966) reacted *in vitro* at pH 5–6 with the guanine of RNA and DNA. Subsequently, Miller *et al.* (1966) showed that the carcinogen *N*-acetoxy-FAA (Miller *et al.*, 1964), another possible *in vivo* metabolite of *N*-hydroxy-FAA (Miller *et al.*, 1966; J. R. DeBaun, J. A. Miller, and E. C. Miller, 1966, unpublished data), reacted readily with guanine in RNA, DNA, and guanosine *in vitro* at pH 7, where *N*-hydroxy-2-fluorenamine had very little activity.

The reaction product of guanosine and *N*-acetoxy-FAA has been identified in the present study as 8-(*N*-2-fluorenylacetamido)guanosine. This structure is analogous to that proposed by Kriek (1965) for the reaction of guanine derivatives with *N*-hydroxy-2-fluorenamine.

Experimental Section

Methods. Thin layer chromatography was carried out on 0.5-mm layers of cellulose MN300 (Brinkmann Instruments, Inc.) in 1-butanol–glacial acetic acid–water (50:11:25) (solvent A) and isopropyl alcohol–concentrated ammonia–water (6:3:1) (solvent B). The cellulose layers were washed by ascension of the solvent to a height of 15 cm, dried under a stream of hot air for 15 min, and then used immediately for chromatography of samples. R_F values are generally given as R_G which is the R_F of the sample relative to that of guanosine. The R_F values of guanosine are 0.33 (solvent A) and 0.50 (solvent B).

The ultraviolet absorption spectra were determined in a Beckman DB spectrophotometer equipped with a Sargent SR recorder. A Zeiss PMQ II spectrophotometer was employed for the pK_a determination. Infrared absorption spectra were determined in a Beckman IR-10 instrument; the compounds were contained in KBr disks. The nuclear magnetic resonance spectra were observed in deuterated dimethyl sulfoxide with Varian A-60 (Nuclear Magnetic Resonance Specialties Co., New Kensington, Pa.; Department of Chemistry, University of Wisconsin) and HA-100 (courtesy of Dr. Norman Bhacca of Varian Associates) spectrometers with tetramethylsilane as an internal standard. The authors are grateful to Mr. John Scribner of the McArdle Laboratory for interpretation of the infrared and nuclear magnetic resonance spectra. Elementary analyses were made by Huffman Laboratories, Wheatridge, Colo.

Reaction of N-Acetoxy-FAA with Guanosine to Yield I. For isolation of the reaction product 120 mg of guanosine monohydrate (Sigma Chemical Co.) (0.40 mmole) in 200 ml of 2 mM sodium citrate buffer at pH 7.0 was mixed with a solution of 600 mg of *N*-acetoxy-FAA (2.14 mmoles) (Lotlikar *et al.*, 1966) in 80 ml of ethanol under nitrogen and incubated at 37° for 3 hr. The reaction mixture was extracted three times with 100-ml portions of ethyl ether, and the aqueous phase was concentrated *in vacuo* at 50° to about 10% of its original volume. The light tan flocculent precipitate that formed was collected on a small suction funnel (Whatman No. 50 paper), washed with a few milliliters of ice-cold water, and dried in air to give about 150 mg of crude product. Crystallization from 30 ml of water gave a white, noncrystalline hygroscopic solid, which was free from guanosine but still contained a small amount of a compound with a lower R_F in solvent A. A second crystallization from 30 ml of water gave 34 mg of chromatographically homogeneous material (I) with R_G values of 2.4 in solvent A and 1.7 in solvent B and with a blue-green fluorescence when viewed under ultraviolet light.

Anal. Calcd for $C_{25}H_{24}N_6O_6 \cdot 0.5H_2O$: C, 58.46; H, 4.92; N, 16.37. Found (dried *in vacuo* over P_2O_5 at 110°): C, 58.95; H, 5.03; N, 16.01.

The elementary analyses and the striking similarity between the ultraviolet absorption spectrum of I (Figure 1) and that of an equimolar mixture of guanosine and FAA (Figure 1) indicated that I was a condensation product of these two compounds. The nuclear magnetic resonance spectra of I suggested that the reaction occurred on the nitrogen atom and not on the fluorene nucleus of *N*-acetoxy-FAA. Integration of the δ 7–8 region in the nuclear magnetic resonance spectra indicated the presence of seven aromatic hydrogen atoms, the correct number for a FAA nucleus with unsubstituted aromatic carbon atoms.

The nuclear magnetic resonance spectrum of guanosine exhibits the absorption of the proton on carbon 8 at δ 8.38 (Gatlin and Davis, 1962). The spectra of I showed no absorption between δ 8 and 10, and thus suggested that substitution had occurred at carbon 8

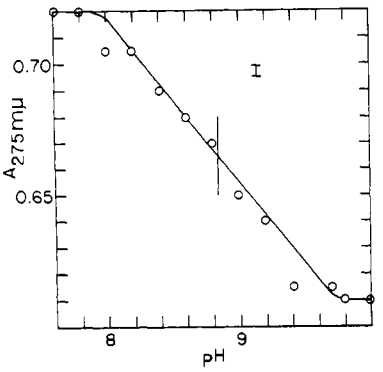

FIGURE 2. The determination of the pK_a of 8-(*N*-2-fluorenylacetamido)guanosine (I); 1.9×10^{-5} M solutions of I in 0.05 M Tris–HCl and sodium carbonate–bicarbonate buffers containing 8% ethanol were employed.

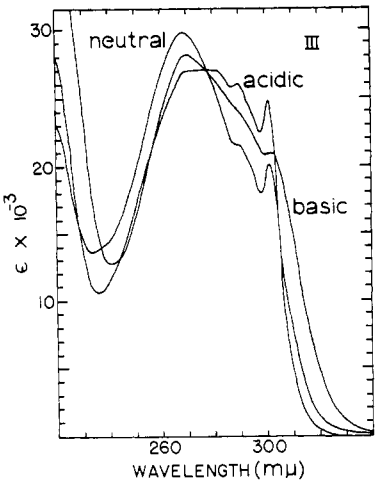

FIGURE 3: Ultraviolet absorption spectra of 8-(*N*-2-fluorenylacetamido)guanine (III) (0.027 mmole/l.) in isopropyl alcohol–water (3:2), isopropyl alcohol–0.25 N HCl (3:2), and isopropyl alcohol–0.025 N sodium hydroxide (3:2).

of guanosine in the reaction with *N*-acetoxy-FAA. Direct evidence for reaction at position 8 was obtained on incubation of guanosine-8-^{3}H (Nuclear Chicago, 0.5 mg, 0.2 μc) with 2.0 mg of *N*-acetoxy-FAA in 1.25 ml of 20% ethanol–2 mM sodium citrate buffer (pH 7) at 37° for 3 hr. Aliquots of the reaction mixture and of a control reaction mixture without *N*-acetoxy-FAA were chromatographed on cellulose in solvent A, and the cellulose fractions were scraped off for determination of radioactivity in a Packard Tri-Carb scintillation counter. Incubation in the presence of *N*-acetoxy-FAA reduced the amount of tritium in the guanosine area to about one-quarter of that present initially, but no tritium was found in the product. Assay of the reaction mixture after drying showed that the tritium from the reacted guanosine had been volatilized, presumably as ^3H_2O.

The reaction product of guanosine and *N*-acetoxy-FAA had a pK_a of approximately 8.8 (Figure 2) as compared to the value of 9.2 for guanosine (Levene and Simms, 1925). This small change is in contrast to the decrease of about 2 units on alkylation of guanosine in the 7 position (Lawley and Brookes, 1961, 1963). No spectral change was found between pH 1 and 7.

Conversion of I to II with Weak Alkali. Compound I (20 mg) was dissolved in 6 ml of 0.01 N NaOH with the aid of a few drops of ethanol, and the mixture was heated at 100° for 30 min. After cooling the solution was neutralized with 0.1 N HCl to pH 7–8. The precipitate which formed was collected by centrifugation, washed with water, and recrystallized from a minimum amount of 50% ethanol. The resulting white noncrystalline solid II (8 mg) was chromatographically homogeneous with R_G values of 1.9 (solvent A) and 1.3 (solvent B). At neutrality the ultraviolet absorption spectrum of II showed a large maximum at 300 mμ and a lesser maximum at 260 mμ (Figure 1). On exposure to ultraviolet light II had a brilliant blue fluorescence and developed a permanent blue-green color.

Reaction of N-Acetoxy-FAA with Deoxyguanosine 5′-Phosphate and the Formation of III. A solution of 400 mg (0.94 mmole) of deoxyguanosine 5′-phosphate disodium salt dihydrate (Sigma Chemical Co.), 200 ml of 2 mM sodium citrate buffer (pH 7), and 1140 mg of *N*-acetoxy-FAA (4.0 mmoles) in 200 ml of ethanol was mixed under nitrogen and incubated at 37° for 3 hr. The reaction mixture was extracted three times with 200-ml portions of ethyl ether, and the aqueous phase was concentrated to 100 ml. Chromatography of the solution on cellulose in solvent B revealed a single reaction product which had a yellow-green fluorescence under ultraviolet light and moved with an R_F of 3.1 relative to that of deoxyguanylic acid. This product was not isolated, but the concentrated aqueous solution was acidified with HCl to a final concentration of 0.05 N. A white flocculent precipitate formed immediately, but the mixture was incubated at 37° for 18 hr to complete the hydrolysis. After collection by centrifugation the precipitate was washed with water, 1% ammonium bicarbonate, and again with water and then dried *in vacuo* over concentrated H_2SO_4 to give 200 mg of a light brown, noncrystalline solid. This material, which had a very low solubility in water, dilute acid, and the common organic solvents was purified by two crystallizations from isopropyl alcohol–water (2:1) to give 20 mg of microcrystalline product III which was chromatographically homogeneous with a R_G value of 2.5 in solvent A.

Anal. Calcd for $C_{20}H_{16}N_6O_2$: C, 64.49; H, 4.34; N, 22.57. Found (dried *in vacuo* at 110° over P_2O_5): C, 65.13; H, 4.66; N, 22.01.

The infrared absorption spectrum of III confirmed the nuclear magnetic resonance spectra of I in that it

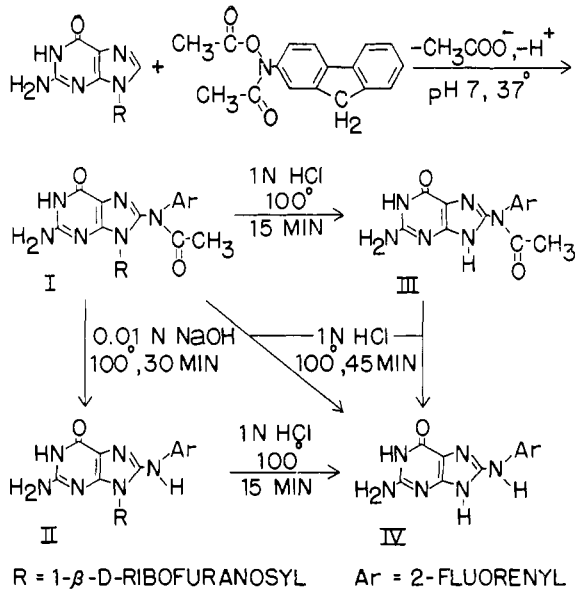

FIGURE 4: The reaction of *N*-acetoxy-FAA with guanosine and the acid and alkaline degradation of the product.

indicated that no substitution had occurred on the aromatic carbons in the FAA nucleus. The maxima observed in the aromatic region of 600–900 cm^{-1} were very similar to those observed for FAA and dissimilar from those noted for 1- and 3-substituted derivatives of FAA (Lotlikar *et al.*, 1966), the most likely possibilities for nuclear substitution.

The ultraviolet absorption spectra of III (Figure 3) showed relatively small shifts over a wide pH range. This behavior contrasts with the large shift in ultraviolet absorption between pH 1 and 7 for 7-substituted guanine derivatives (Brookes and Lawley, 1961; Colburn *et al.*, 1965).

Hydrolysis of I, II, and III to IV. To study the time course of hydrolysis of I and III solutions of these compounds in 1 N HCl (1 mg/ml) were immersed in a boiling water bath, and aliquots were removed at 15-min intervals for chromatography on cellulose. After 15 min I appeared to be completely hydrolyzed to ribose (detected by the aniline–phthalate reagent (Partridge, 1949) after chromatography in the 1-butanol–acetic acid–water system of Partridge (1948)) and two fluorene derivatives. One was identical with III in its chromatographic behavior and ultraviolet spectrum, while the other (IV) had a brilliant blue fluorescence when viewed under ultraviolet light and had a R_G value of 1.5 (solvent A). After 45 min in 1 N HCl at 100° both I and III were completely converted to IV which was stable upon further treatment with 1 N HCl. Similarly, hydrolysis of II in 1 N HCl at 100° for 15 min liberated both ribose and IV.

Synthesis of 8-(N-2-Fluorenylamino)guanine and Its Identity with IV. A suspension of 0.20 g (0.41 mmole) of 8-bromoguanosine 2′,3′,5′-triacetate (Aldrich Chemical Co.) and 0.36 g (2.0 mmoles) of 2-fluorenamine (Kuhn, 1943) in 5 ml of isopropyl alcohol was flushed with nitrogen gas, frozen in liquid nitrogen, and sealed *in vacuo* in a 10-ml thick-walled glass tube. The tube was heated at 150° for 20 hr, cooled, and opened after the contents were frozen in liquid nitrogen. The brown precipitate was filtered and washed with five 2-ml portions of methanol. This precipitate (0.23 g) was refluxed for 1 hr in 150 ml of ethanolic (50%) 1 N HCl and filtered by suction immediately after boiling ceased. The filtrate was diluted with two volumes of 1 N HCl in water and, upon cooling to room temperature, the voluminous white precipitate that formed was collected by centrifugation and again dissolved in hot ethanolic HCl and reprecipitated in the same manner. The latter precipitate was dissolved in a minimum volume of hot 9:1 95% ethanol–concentrated HCl, and crystallization occurred at 5°. Light yellow crystals (0.037 g, 23% yield) of 8-(*N*-2-fluorenylamino)guanine monohydrochloride monohydrate were obtained.

Anal. Calcd for $C_{18}H_{14}N_6O \cdot HCl \cdot H_2O$: C, 56.18; H, 4.45; Cl, 9.21; N, 21.84; O, 8.32. Found (dried *in vacuo* at 60° over P_2O_5): C, 56.30; H, 4.52; Cl, 9.28; N, 21.77; O, 8.53.

Compound I was hydrolyzed to IV in 1 N HCl in 50% ethanol at reflux temperature for 1 hr. The hydrolysis mixture was cooled and diluted with 1 N HCl in water, and the precipitate was crystallized as described above for 8(-*N*-2-fluorenylamino)guanine. The crystalline product IV was identical with the latter compound with respect to its ultraviolet absorption spectrum (Figure 1), infrared absorption spectrum (maxima at 3340, 3130, 2900, 1655, 1620, 1595, 1520, 1450, 1400, 1290, 1250, 1205, 1135, 1020, 950, 875, 820, 760, 720, 680, 660, 615, 515, 420, and 360 cm^{-1}), and chromatography on cellulose in solvent A. Compound IV and 8-(*N*-2-fluorenylamino)guanine also exhibited the same blue fluorescence and the same lability to ultraviolet light when the free base was exposed.

Identification of I–III. Figure 4 shows the structure assignments for I–III on the basis of the following facts. All three compounds can be converted to 8-(*N*-2-fluorenylamino)guanine (IV) by hydrolysis and must therefore contain this nucleus in common. Acid hydrolysis of I or II liberates ribose; these derivatives must therefore be ribosides. The molecular formula of I is consistent with the structure 8-(*N*-2-fluorenylacetamido)guanosine; II, formed from I by weak alkali, must, therefore, be 8-(*N*-2-fluorenylamino)guanosine. The molecular formula of III agrees with that of 8-(*N*-2-fluorenylacetamido)guanine, and this structure is consistent with the formation of III as an intermediate in the acid hydrolysis of 8-(*N*-2-fluorenylacetamido)guanosine to 8-(*N*-2-fluorenylamino)guanine. Furthermore, the product of the reaction of deoxyguanosine 5′-phosphate and *N*-acetoxy-FAA can be identified as 8-(*N*-2-fluorenylacetamido)deoxyguanosine 5′-phosphate by analogy with the reaction of guanosine and *N*-acetoxy-FAA and because of the degradation

of the deoxyguanylic acid product with weak acid to 8-(*N*-2-fluorenylacetamido)guanine.

The neutral ultraviolet absorption spectra in Figures 1 and 3 lend further support to these assignments. Compounds I and III, the two derivatives with *N*-acetyl groups, have absorption maxima at 275 and 268 mμ, respectively, and a minor peak or shoulder at 300 mμ. Compounds II and IV, the two fluorenylamino derivatives, have their major maxima strongly shifted to longer wavelengths, 300 mμ in the case of 8-(*N*-2-fluorenylamino)guanosine and 332 mμ for 8-(*N*-2-fluorenylamino)guanine. The latter two derivatives have a brilliant blue fluorescence and are labile to ultraviolet light with the development of a blue-green color. The acetamido derivatives emit a bluish-green fluorescence.

Stability of 8-(N-2-Fluorenylacetamido)deoxyguanosine 5′-Phosphate. Unlike the 7-alkylated derivatives of deoxyguanosine 5′-phosphate (Lawley, 1957; Brookes and Lawley, 1960; Lawley and Brookes, 1963) 8-(*N*-2-fluorenylacetamido)deoxyguanosine 5′-phosphate was stable at neutrality. Thus, after heating a concentrated reaction mixture which contained deoxyguanylic acid and 8-(*N*-2-fluorenylacetamido)deoxyguanosine 5′-phosphate for 1 hr at pH 7 at 100°, chromatography on cellulose in solvent B still revealed only the presence of these two compounds. In another study no degradation was observed on maintaining 8-(*N*-2-fluorenylacetamido)deoxyguanosine 5′-phosphate or deoxyguanylic acid at 37° for 6 days at pH 7.2, while under the same conditions a major share of 7-methyldeoxyguanosine 5′-phosphate was degraded to 7-methylguanine within 1 day (Figure 5). The 8-(*N*-2-fluorenylacetamido)deoxyguanosine 5′-phosphate for the latter experiment was prepared by reacting 20 mg of deoxyguanosine 5′-phosphate in 7 ml of 3.3 mM pH 7.2, sodium phosphate buffer with 60 mg of *N*-acetoxy-FAA in 6.0 ml of ethanol under a nitrogen atmosphere for 3.5 hr at 37°, removing most of the ethanol *in vacuo*, and then extracting the solution four times with equal volumes of ethyl ether. The 7-methyldeoxyguanosine 5′-phosphate was prepared by reaction of 20 mg of deoxyguanosine 5′-phosphate in 4 ml of 133 mM, pH 7.2, sodium phosphate buffer with 100 mg of dimethyl sulfate in 1 ml of ethanol for 2 hr at 37°. Each of these solutions and a solution of 20 mg of deoxyguanosine 5′-phosphate was then diluted to a final volume of 12 ml which contained 40% of ethanol and was 44 mM with respect to sodium phosphate buffer at pH 7.2. These solutions were maintained at 37°, and aliquots were removed at intervals for chromatography on cellulose thin layers. The chromatograms of 8-(*N*-2-fluorenylacetamido)deoxyguanosine 5′-phosphate were developed in solvent B, while those of 7-methyldeoxyguanosine 5′-phosphate or of deoxyguanosine 5′-phosphate were developed in a system composed of saturated aqueous ammonium sulfate–isopropyl alcohol–33 mM sodium phosphate buffer, pH 7.2 (72:2:19) (Lawley, 1957). In each case the nucleotide was eluted with 33 mM, pH 7.2, phosphate buffer and quantitated spectrophotometrically.

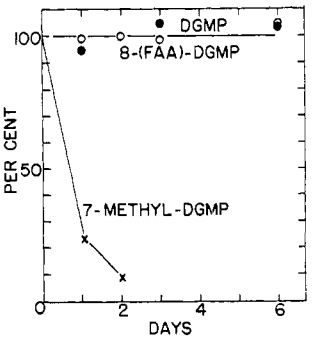

FIGURE 5: The stabilities of 7-methyldeoxyguanosine 5′-phosphate (7-methyl-DGMP) (×), 8-(*N*-2-fluorenylacetamido)deoxyguanosine 5′-phosphate (8-(FAA)-DGMP) (○), and deoxyguanosine 5′-phosphate (●) at pH 7.2 and 37°.

Reaction of N-Acetoxy-FAA with Guanine Bases in Nucleic Acids in vitro. Both sRNA and DNA, after reaction with *N*-acetoxy-FAA at pH 7 *in vitro*, yield polynucleotides with increased ultraviolet absorption in the region of 280–320 mμ and with decreased contents of guanine (Miller *et al.*, 1966). Each of these altered polynucleotides also yielded upon acid hydrolysis an acid-insoluble, ethanol-soluble product (Miller *et al.*, 1966). This product had the same ultraviolet absorption spectrum (Miller *et al.*, 1966), infrared absorption spectrum, and R_G on cellulose in solvent A as those of IV or 8-(*N*-2-fluorenylamino)guanine. Similarly, the product showed a bright blue fluorescence and developed a blue-green color upon exposure to ultraviolet light. Thus, *N*-acetoxy-FAA reacted with the guanine bases in DNA and RNA in the same fashion as observed above for guanosine or deoxyguanosine 5′-phosphate.

Discussion

The ability of *N*-acetoxy-FAA to arylamidate or attach the nitrogen of a FAA residue to the 8-carbon of guanine in guanosine, deoxyguanosine 5′-phosphate, and nucleic acids *in vitro* is of particular importance since this reaction may be the prototype of that which occurs between metabolites of FAA and tissue nucleic acids *in vivo* (Farber *et al.*, 1962; Marroquin and Farber, 1962, 1965; Williard and Irving, 1964; Miller *et al.*, 1964; Sporn and Dingman, 1966). Other data from this laboratory (Lotlikar *et al.*, 1966; J. R. DeBaun, J. A. Miller, and E. C. Miller, 1966, unpublished data) point to the formation *in vivo* of a derivative of FAA with reactivity toward hepatic protein-bound methionine identical with that observed *in vitro* between *N*-acetoxy-FAA and methionine or methionyl peptides. Furthermore, preliminary studies show that the major share of the radioactivity released by alkaline phosphatase and diesterase from rat liver ribonucleic acids isolated after administration of *N*-hydroxy-FAA-9-^{14}C *in vivo* moves in solvent A with the same mobility

as 8-(*N*-2-fluorenylacetamido)guanosine and faster than 8-(*N*-2-fluorenylamino)guanosine (E. C. Miller, J. A. Miller, and U. Juhl, 1966, unpublished data).[2]

It is of interest to compare some of the possible consequences of the reaction of *N*-acetoxy-FAA at carbon 8 of guanine in nucleic acids with those of the well-known attack of alkylating agents at nitrogen 7 of this base (Lawley, 1957; Brookes and Lawley, 1961, 1964). Substitution in the 8 position does not lead to the instability of the deoxyribosidic linkage to hydrolysis at neutrality that is noted after substitution in the 7 position (Lawley, 1957; Brookes and Lawley, 1960; Lawley and Brookes, 1963). Thus, if these fluorenyl derivatives of DNA-guanine are formed *in vivo* they might be relatively stable components of DNA. Furthermore, substitution in the 8 position of guanosine with FAA does not greatly increase acidic ionization at the 1-nitrogen, as evidenced by the small drop in the pK_a. The alkylation of guanine derivatives at position 7 considerably increases acidic ionization at the 1-nitrogen and thereby possibly causes *in vivo* a mispairing of alkylated guanine with thymine in DNA (Lawley and Brookes, 1961; Nagata *et al.*, 1963). These and other aspects of the attachment of a bulky molecule such as FAA to the 8-carbon of guanine in the DNA double helix and in RNA deserve further study in view of their possible roles in carcinogenesis by FAA and its carcinogenic metabolites.

Acknowledgment

The authors are grateful to Miss Carol Rasmussen for assistance in studies on the stabilities of the derivatives of deoxyguanosine 5′-phosphate.

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[2] Irving *et al.* (1966) observed that administration to rats of either *N*-hydroxy-FAA-9-^{14}C (ring labeled) or *N*-hydroxy-FAA-1′-^{14}C (*N*-acetyl labeled) gave equal amounts of radioactivity bound to the liver RNA. The *N*-acetyl group is thus retained in the binding of the fluorene metabolite to the RNA. We are indebted to Dr. Irving for the privilege of seeing this manuscript prior to publication.