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Reaction of Acrolein with Cytosine and Adenine Derivatives

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The products of reaction of acrolein with the following cytosine and adenine derivatives have been characterized: 1-methylcytosine, cytidine, deoxycytidine, 9-methyladenine, adenosine, and deoxyadenosine. The products contain new six-membered rings, formed by Michael addition of the amino group of cytosine or adenine to the carbon–carbon double bond of acrolein and addition of ring nitrogen 3 of cytosine or 1 of adenine across the acrolein carbonyl group. The orientation of addition was established by oxidation of the adduct of 1-methylcytosine and acrolein to N^4-(2-carboxyethyl)-1-methylcytosine and that of 9-methyladenine and acrolein to N^6-(2-carboxyethyl)-9-methyladenine. Cyclic adducts of this type, if formed in DNA *in vivo*, may contribute to the genetic effects of acrolein. © 1988 Academic Press, Inc.

Acrolein is a significant environmental pollutant, as it occurs in tobacco smoke and automobile exhausts. It is formed when animal and vegetable fats are heated, as in frying processes. In addition it has many industrial uses in the synthesis of drugs, plasticizers, and other substances of commercial importance (*1*). Biologically, acrolein exhibits a number of adverse effects, serving as a respiratory irritant, cytotoxic substance, and cell growth inhibitor (*1, 2*). Mutagenic effects have been noted in *Salmonella* (*2–4*) and *Drosophila* (*1, 2*), but other workers have obtained negative results in these systems and the situation has been called "conflicting and contradictory" (*2*). The same review concluded that there was "insufficient data available for evaluation" of the carcinogenic potency of acrolein.

Because of the possible genetic effects of acrolein, one reviewer considered its reactions with nucleic acids "the most dangerous perhaps, among the many interactions attributable to its size and reactivity" (*1*). Over the years, a number of workers have reported spectroscopic observations or other descriptions of reactions of acrolein with nucleic acids or their components (*1, 3, 5, 6*), but only one group has characterized reaction products and assigned definite structures to them: Chung *et al.* reported that cyclic, $1,N^2$-adducts are formed by reaction of acrolein with deoxyguanosine and with guanine in DNA (*7*). The possibility of adduct formation with other DNA bases was not excluded, but was not examined. Cyclic DNA adducts derived from a number of other reagents have come into prominence as mutagens and carcinogens (*8*), and the above authors (*7*) suggested that the guanine adducts, or those derived from other bases, may play a role in

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acrolein mutagensis. We now describe the characterization of cyclic adducts formed by reaction of acrolein with adenine and cytosine nucleosides (a preliminary account of these results has been given (*9*)). The broader reactivity demonstrated for acrolein increases the plausibility of claims of its significant genetic effects.

METHODS

Ultraviolet spectra were measured on a Cary 15 spectrophotometer. Reported pK_a values were determined (*10*) using this instrument. The wavelengths employed were 286 nm for **I** and **III**, 260 nm for **IV**, and 270 nm for **VI**. The buffers used were 0.01 M sodium or potassium phosphate, acetate, 4-aminovalerate, and borate. The pH values were measured using a Radiometer pHM62 pH meter equipped with a combined electrode of type GK2322C. Proton NMR spectra (100 MHz) were obtained with a Varian XL-100 spectrometer equipped with a Nicolet Fourier transform accessory. Mass spectra (electron impact) were obtained using a Dupont Model 24-49 double focusing mass spectrometer. Elemental analyses were performed by Spang microanalytical laboratory, Eagle Harbor, Michigan. Analytical silica gel and Avicel plates for thin layer chromatography were purchased from Brinkmann and Instruments, and preparative plates of the same materials were employed for TLC: a, 2-propanol/water/concd NH_4OH (7/2.5/1.5, v/v); b, 2-propanol/water (7/3, v/v); c, butanol/water (86/14, v/v). Amberlite ion exchange resins were obtained from Mallinkrodt and Dowex resins from Bio-Rad Laboratories. Acrolein was obtained from Eastman Kodak and was freshly distilled before use.

Reaction of 1-methylcytosine with acrolein: Preparation of ***Ia.*** 1-Methylcytosine (1.5 g) and acrolein (0.9 ml) were allowed to react for 36 h under N_2 in 240 ml of 1.0 M sodium acetate buffer, pH 4.37, 25°C. Unreacted acrolein was removed by passing a N_2 stream through the reaction mixture for 4 h. The mixture was concentrated under vacuum to produce a viscous oil. Absolute ethanol was added, and the resulting precipitate removed by filtration and discarded. The filtrate was concentrated under vacuum, and the ethanol precipitation procedure was repeated two times. The final oil was evaporated three times with chloroform and then allowed to crystallize at −10°C from chloroform in several crops. Recrystallization of the final product from chloroform afforded 600 mg (26%) of **Ia** (acetate salt) as white crystals: mp 131–132°C; R_f (system a) = 0.67; ^1H NMR (D_2O): δ = 7.55, d, 1H (H-6); 5.95, d, 1H (H-5); 5.15, t, 1H (NC*H*OH); 3.57–4.33, m, 2H (NCH_2); 3.36, s, 3H (NCH_3); 1.90–2.20, m, 2H (CCH_2C); 1.85, s, 3H (CH_3COO^-); uv, λ_{max}: pH 2.8, 215 and 285 nm; pH 7.3, 215 and 285 nm; pH 12.0, 230 and 278 nm; pK_a 8.98.

The acetate salt of **Ia** was converted to the free base by passage through a column of Dowex I-X8 anion exchange resin (HCO_3^- form). Evaporation of the eluate afforded white crystals (decomp 153–154°C). ^1H NMR: δ = 7.02, d, 1H (H6); 5.61, d, 1H (H5); 4.96, t, 1H (NC*H*OH); 3.60–3.90, t, broad, 2H, (NCH_2);

3.20, s, 1H (NCH_3); 1.70–2.10, broad, 2H (CCH_2C). *Anal.* Calcd for $C_8H_{11}O_2N_3$: C, 53.03; H, 6.10; N, 23.19. Found: C, 53.07; H, 6.03; N, 23.12.

Oxidation of ***Ia*** *to* N^4*-(2-carboxyethyl)-1-methylcytosine* (***III***). To 100 mg of **Ia** in 2 ml of aqueous 5% KOH solution was added 130 mg of silver oxide. The mixture was heated at 70°C for 4.5 h and then acidified with 6 N HCl and evaporated to dryness. The residue was extracted with ethanol and filtered. The filtrate was concentrated and worked up by preparative TLC (solvent a). The band of R_f 0.65, eluted with water, afforded 65 mg (60%) of **III:** ^1H NMR (D_2O): δ = 7.49, d, 1H (H6); 5.90, d, 1H (H5); 3.55, t, 2H ($-NCH_2$); 3.35, s, 3H (NCH_3); 2.55, t, 2H ($-CH_2CO$). uv, λ_{max}: pH 2.8, 285 nm; pH 6.7 and 10.7, 275 nm; pK_a 3.93. A minor product, 5 mg, was obtained by eluting the material in a band of R_f 0.81: uv, λ_{max}: pH 1 and 7, 266 nm; pH 12, 263 nm. The uv spectra resembled those of a 1-substituted uracil. This product was also formed from **Ia** in a control reaction lacking silver oxide.

Reaction of cytidine with acrolein: Preparation of ***Ib***. The procedure used for the preparation of **Ia** was followed, using 1.5 g of cytidine and 0.6 ml of acrolein. After removal of unreacted acrolein, the final reaction mixture was concentrated to 20 ml and passed through a column of Dowex I-X8, eluting with water. The first ultraviolet-absorbing material to emerge was collected and concentrated to about 2 ml, and absolute ethanol was added. The white precipitate which appeared (non-ultraviolet absorbing) was discarded. The filtrate was concentrated under vacuum, and the ethanol precipitation repeated, for a total of four times. Ether was then added to the final concentrated solution to precipitate the product. The product was washed with ether and finally crystallized from cold absolute ethanol (yield 0.52 g, 29%). The white crystals of **Ib** softened on heating at 146°C and decomposed at 200°C: ^1H NMR (D_2O): δ = 7.27, d, 1H (H6); 5.80, d, 1H (H1′); 5.70, d, 1H (H5); 4.98, t, 1H (NC*H*OH); 3.85–4.25. m, 7H (NCH_2, H2′, H3′, H4′, H5′, H5″); 1.70–2.15, broad, 2H (CCH_2C). uv, λ_{max}: pH 2.3 and 6.8, 282 nm; pH 10.4, 230 and 271 nm; pK_a 8.27. *Anal.* Calcd for $C_{12}H_{17}N_3O_6$: C, 48.16; H, 5.73; N, 14.04. Found: C, 47.38; H, 5.67: N, 13.64.

Reaction of deoxycytidine and acrolein: Preparation of ***Ic***. The reaction and workup were conducted as described for **Ib** above, using 340 mg of deoxycytidine and 0.1 ml of acrolein in 40 ml of 0.1 M acetate buffer. The eluate from the Dowex I-X8 column was analyzed by TLC, fraction by fraction. The later portions of the eluate from the first ultraviolet-absorbing peak, which contained unreacted deoxycytidine as well a product, were set aside. The product-containing solution was concentrated, evaporated repeatedly first with absolute ethanol and then with ethyl acetate, and finally crystallized from cold ethyl acetate. The white crystals of **Ic** were washed with ether and dried under vacuum: 100 mg, 45%, mp 152–153°C. ^1H NMR: δ = 7.27, d, 1H (H6); 6.20, t, 1H (H1′); 5.73, d, 1H (H5); 4.99, t, 1H (NC*H*OH); 4.4, dd, 1H (H4′); 3.6–4.5, m, 5H (NCH_2, H3′, H5′, H5″); 2.3, m, 2H (H2′, H2″); 1.70–2.15, broad, 2H (CCH_2). uv, λ_{max}: pH 4.2 and 7.1, 282 nm; pH 10.8, 230 and 270 nm; pK_a 8.52. *Anal.* Calcd for $C_{12}H_{17}N_3O_5$: C, 50.88; H, 6.05; N, 14.83. Found: C, 50.76; H, 6.02; N, 14.74.

Reaction of 9-methyladenine and acrolein: Preparation of IVa. 9-Methyladenine (55 mg) and 0.035 ml of acrolein were combined under nitrogen in 17 ml of

0.1 M sodium acetate buffer, pH 4.1, and allowed to react at 25°C for 48 h. Unreacted acrolein was removed with a stream of nitrogen gas and the solution was evaporated under vacuum. The residue was dissolved in water and chromatographed on a column of Amberlite CG-50, H^+ form, eluting with 1% acetic acid. The ultraviolet-absorbing material that emerged was evaporated under vacuum and rechromatographed on a column of Dowex I-X8 resin, Cl^- form, eluting with water. The ultraviolet-absorbing material was collected, concentrated to 2 ml, and crystallized by addition of acetone and cooling. White crystals of **IVa** hydrochloride (17 mg, 19%) were collected. ^1H NMR (D_2O): δ = 8.54, s, 1H (H2 or H8); 8.24, s, 1H (H2 or H8); 5.50, t, 1H (NC*H*OH); 4.30–4.70, m, 2H (NCH_2); 3.89, s, 3H (NCH_3); 2.35, m, 2H, (CCH_2C). uv, λ_{max}: pH 2.75, 264.5 nm; pH 11.3, 276 nm; pK_a 9.45. *Anal.* Calcd for $C_9H_{12}N_5OCl$: C, 44.73; H, 5.01; N, 28.98; Cl, 14.67. Found: C, 44.65; H, 4.97; N, 28.88; Cl, 14.63.

*Oxidation of **IVa** to N^6-(2-carboxyethyl)-9-methyladenine (**VI**).* To 100 mg of **IVa** in 30 ml of aqueous 2% KOH solution was added 140 mg of silver oxide, and the reaction mixture was heated at 60°C for 15 min. The mixture was centrifuged, and the supernatant acidified with HCl and centrifuged again. The solution was evaporated under vacuum, and the residue dissolved in 8 ml of water. Addition of 8 ml of ethanol afforded a precipitate of non-ultraviolet-absorbing material which was separated by filtration. The filtrate was evaporated to dryness, affording 70 mg (76%) of **VI** as a crystalline solid, mp 237–245°C, which was homogeneous to TLC (solvent b). The substance migrated toward the anode upon paper electrophoresis at pH 7.45 in 0.1 M potassium phosphate buffer. ^1H NMR (D_2O): δ = 8.70, s, 1H (H2 or H8); 8.55, s, 1H (H2 or H8); 4.14, s (NCH_3) superimposed upon 4.14–4.50, broad (NCH_2); 3.15, t (CH_2CO). uv, λ_{max}: pH 2.0, 264 nm; pH 7.5 and 11.5, 267.5 nm; apparent pK_a 3.72; mass spectrum, *m/z* 221 (M^+), 176 (base), 149, 133.

*Reaction of adenosine and acrolein: Preparation of **IVb**.* The reaction was conducted and worked up as described for the preparation of **IVa** above, using 267 mg of adenosine and 0.075 ml of acrolein in 50 ml of acetate buffer. The eluate from the Dowex I-X8 column was evaporated under vacuum, taken up in absolute ethanol, and precipitated with ether to afford 200 mg (62%) of **IVb** hydrochloride as a white powder. ^1H NMR (D_2O): δ = 8.57, s, 2H (H2 + H8); 6.10, d, 1H (H1′); 5.50, t, 1H (NC*H*OH); 4.20–4.90, m + solvent peak (NCH_2, H2′, H3′ or H4′); 4.10, dd, 1H, H3′ or H4′; 3.85, m, 2H (H5′, H5″); 2.35, dd (broad), 2H, CCH_2C). uv, λ_{max}: pH 2.9 and 6.5, 261 nm; pH 10.6, 265 nm; pK_a 9.05. *Anal.* Calcd for $C_{13}H_{18}N_5O_5Cl$: C, 43.40; H, 5.04, N, 19.47; Cl, 9.86. Found: C, 43.20; H, 5.04, N, 19.37; Cl, 9.87.

In an alternative workup, a reaction mixture prepared as above, but on one-fifth the scale, was applied to a column of Amberlite CG-50, H^+ form, rather than to the Dowex I column. The Amberlite column was washed with water and then with 1% acetic acid to elute the product. The solvent was evaporated, affording 50 mg of a white solid whose properties were the same as those of **IVd** (below).

*Reaction of deoxyadenosine and acrolein: Preparation of **IVc**.* The reaction was conducted as described for **IVa**, using 500 mg of deoxyadenosine and 0.175 ml of acrolein in 100 ml of acetate buffer. After removal of acrolein, the reaction

mixture was applied to a column of Dowex I X-8 resin, Cl^- form, and eluted with water. The material within the first half of the ultraviolet-absorbing peak was uncontaminated product, by TLC analysis. This fraction was evaporated under vacuum, dissolved in absolute ethanol, and precipitated with acetone. Compound **IVc** was obtained as a white powder (200 mg, 34%). ^1H NMR (D_2O): δ = 8.50, s, 2H (H2 + H8); 6.50, t, 1H (H1′); 5.45 t, 1H (NC*H*OH); 4.25–4.75, m + solvent peak (NCH_2, H3′ or H4′); 4.10, dd, 1H (H3′ or H4′); 3.75, d, 2H (H5′, H5″); 2.40–2.95, m, 2H (H2′, H2″), 2.35, dd (broad), 2H, CCH_2C). uv, λ_{max}: pH 2.1, 261 nm, pH 11.0, 265 nm; pK_a 9.09.

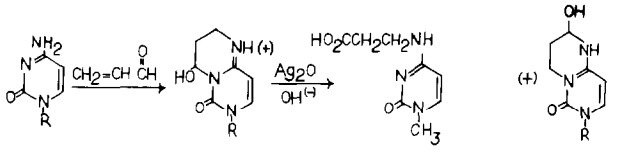
*Reaction of adenine and acrolein: Formation of **IVd**.* The reaction was conducted as described for **IVa,** using 500 mg of adenine and 0.33 ml of acrolein in 90 ml of acetate buffer. After 48 h of reaction, the reaction mixture was concentrated under vacuum to a small volume and subjected to preparative TLC on silica gel in solvent b. New ultraviolet-absorbing bands of R_f 0.46 (major product) and 0.67 were observed. The latter band decomposed in part to a substance with the mobility of adenine upon attempted rechromatography. The material in the 0.46 band was eluted with methanol–water (1 : 1) and rechromatographed on Avicel cellulose in solvent c to afford **IVd** (150 mg, 16%) as its acetate salt. ^1H NMR (D_2O): δ = 8.15, s, 1H (H2 or H8); 7.99, s, 1H (H2 or H8); 5.38, t, 1H (NC*H*OH); 4.20–4.55, m, 2H (NCH_2); 2.25–2.35, m, 2H (CCH_2C); 2.0, s (CH_3COO^-). uv, λ_{max}: pH 2.9, 262 nm; pH 11.1, 274 nm; pK_a 6.88 and >11.

The above reaction was repeated at pH 7.0 (0.1 M sodium phosphate buffer) for 60 h, on $\frac{1}{30}$ the scale. Two bands, R_f 0.46 and 0.67 (major product), were observed upon preparative TLC on silica gel, solvent b. The bands were extracted using ethanol, and the solvent was evaporated under vacuum. The spectroscopic properties of the band of R_f 0.46 were the same as those of the major pH 4.1 product. The product from the band of R_f 0.67 was crystallized from an ethanol–chloroform–ether mixture to afford 7 mg of white crystals. ^1H NMR (CD_3SOCD_3): δ = 7.80, s, 1H (H2 or H8); 7.70, s, 1H (H2 or H8); 4.70, m, 2H; 3.80–4.20, m, 4H (NCH_2); 1.70–2.10, m, 4H (CCH_2C). uv, λ_{max}: pH 2.1, 280 nm, 290 nm (sh); pH 5.8, 278 nm, 272 nm (sh), 288 nm (sh); pK_a 3.78.

RESULTS

Reaction of Acrolein with 1-Substituted Cytosines

The reactions of acrolein with 1-methylcytosine, cytidine, and deoxycytidine were examined at several pH values using differing ratios of reactants. The use of a pH near 4, with a slight excess of acrolein, seemed optimal for preparative purposes. Under those conditions, the reaction went largely to completion, with only one new product observed by thin layer chromatography. The use of greater excesses of acrolein led to the appearance of additional substances which, on the basis of their NMR spectra, appeared to involve more than one acrolein for each cytosine. They were not investigated further. The reactions proceeded much more slowly at pH 7 than in acid (only a small amount of conversion after 24 h, 25°C),



a) R = CH_3
b) R = ribosyl
c) R = deoxyribosyl

SCHEME I

although the same products were formed. In alkaline solution, side reactions of acrolein predominated. The workup generally involved preparative thin layer chromatography. In some cases, the separation of product (first peak eluted) from unreacted cytosine derivative could be achieved by chromatography on Dowex I-X8 resin.

The products, **Ia–Ic** (Scheme I), exhibited similar properties. They analyzed as 1 : 1 adducts of acrolein and the cytosine derivative. Their pK_a values (8.98, 8.27, and 8.52 for **Ia–Ic**) and ultraviolet spectra at various pH values were characteristic of 1,3- or 1,3,N^4-substitution on cytosine (*11*). The ^{1}H NMR spectra, in D_2O, indicated the presence of the methyl or sugar substituent, H5 and H6 of cytosine, and three new groups of protons, in a ratio of 1 : 2 : 2, derived from acrolein. Their chemical shifts were incompatible with the presence of vinyl or aldehydic protons and suggested that cyclic adducts, such as **I** or **II,** had been formed. The structure of the peaks representing the CCH_2C and NCH_2C protons exhibited a pronounced dependence on pD. This phenomenon was studied most closely for the 1-methylcytosine product. At alkaline pD, the cited peaks were broad singlets, centered at about δ 1.95 and 3.80, respectively. In DCl solution, however, the former peak took on considerable fine structure while the latter split into two separate well-defined multiplets, each integrating as one proton, centered at δ 3.72 and 4.28.

One possible explanation of this behavior is the following: In structure **I** or **II,** the component protons of the CCH_2 and NCH_2 groups would have distinct chemical identities, determined by their stereochemistry *cis* or *trans* to the ring hydroxyl group. The coalescence and loss of fine structure of their ^{1}H NMR spectra in alkali suggests a base-catalyzed interconversion of the *cis*- or *trans*-protons, perhaps through an intermediate open chain aldehyde form. When the NMR spectrum of the 1-methylcytosine–acrolein adduct was taken an strong base (pD 12), a small peak corresponding in shift to an aldehyde proton was in fact observed (the compound rapidly decomposed under these conditions, however). Alternatively, the behavior observed in the NMR spectra may result from the presence of interconverting conformers in the unprotonated form of **I** and a rigid system in the protonated form.

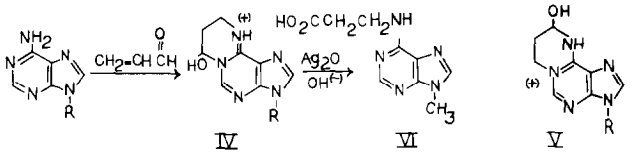
The first explanation suggested a method for distinguishing between the alternative structures, **Ia** and **IIa.** Oxidation under alkaline conditions could convert the

adduct, perhaps through the aldehyde intermediate, to a stable derivative that could readily be identified. When a procedure of this type was carried out, the major product was a carboxylic acid. It was identical in its spectroscopic and chromatographic properties to the known compound N^4-(2-carboxyethyl)-1-methylcytosine, which was synthesized by an alternative, established route (*12*). This result indicated that **Ia,** rather than **IIa,** was the structure of the 1-methylcytosine–acrolein product. By analogy, and because of the similarity in properties, structures **Ib** and **Ic** were assigned to the cytidine and deoxycytidine adducts. (These adducts are capable of existing as mixtures of diastereoisomers, but the available chromatographic and NMR data showed no evidence of this.)

Reaction of Acrolein with 9-Substituted Adenines

Adenine bears a structural resemblance to cytosine in that each has an amino group adjacent to a pyridine-type ring nitrogen within a pyrimidine ring. The reactions of 9-methyladenine, adenosine, and deoxyadenosine with acrolein also had much in common with those of their cytosine counterparts. The considerations regarding optimal pH and other reaction conditions were the same, and the same workup procedures were followed. A series of 1 : 1 adducts were obtained, **IVa–IVc** (Scheme II). The pK_a values (9.45, 9.05, and 9.09, respectively) and ultraviolet spectra of the adducts were consistent with a 1,9- or 1,9,N^6-substitution pattern on adenine (*10*). The pattern of peaks occurred in the ^{1}H NMR spectra were quite similar to those in the cytosine series and indicated a cyclic structure, **IV** or **V,** for the adducts. To help distinguish these possibilities, **IVa** was subjected to the alkaline oxidation procedure used for **Ia.** A single product was again obtained, whose pK_a, ultraviolet, ^{1}H NMR, and mass spectrum and electrophoretic mobility were consistent with structure **VI,** Scheme II. If we presume that the formation of **VI** took place by a straightforward oxidation process, then **IVa** may be assigned as the structure of the 9-methylcytosine–acrolein adduct and, by analogy, **IVb** and **IVc** as the structures of the nucleoside adducts.

One ambiguity, not present in the cytosine series, remains in this identification. 1-Substituted adenines rearrange under alkaline conditions to the N^6-substituted derivates in the Dimroth rearrangement (*13*). For 1-carboxyethyl-9-substituted adenines, the process is complete at pH 11, 19 h, 25°C (*14*) and at pH 11.7, 18 h,



a) R = CH_3
b) R = ribosyl
c) R = deoxyribosyl
d) R = H

SCHEME II

37°C (*15*). Our oxidation of **IXa** to **Xa** was complete after 15 min, 60°C, at a pH 13. An extrapolation from published kinetic data on 1,9-dialkyladenines (*16*) suggests that the Dimroth reaction should require only a few minutes under our conditions. No intermediate was observed by TLC during the reaction, but if the rearrangement proceeded more rapidly than the oxidation, which is conceivable, observation of the 1-carboxyethyl intermediate would not be expected. The tentative assignment of structures **IV** to the acrolein reaction products in the adenine series rests then on the resemblance of these products to the cytosine series in their ^{1}H NMR and the presumption that no Dimroth rearrangement took place in the formation of **VI.**

Reaction of Acrolein with Adenine

The above studies were conducted to gain insight into the reactions of acrolein with nucleosides, and ultimately with DNA and RNA. We examined the reaction of acrolein and adenine with another purpose in mind. It has been suggested that acrolein would interact with the bases of RNA by simple Michael addition, with 9-substitution in the case of the purines and 1-substitution in the case of the pyrimidines (*5, 17*). These adducts would then undergo further transformations of interest to prebiotic chemistry (see Discussion). These adducts had not been characterized, however, so we sought to do so for the case of adenine. The major product of a reaction run at pH 4.1 had properties which resembled those produced from 9-substituted adenines. Further, it could be produced by treatment of the deoxyadenosine product **IVc** with acid. Structure **IVd** was therefore assigned to this substance. A minor product produced at pH 4.1 readily reverted to adenine and was not characterized. The adenine reaction took a different course when run at pH 7.0. Product **IVd** was formed slowly and was a minor reaction product, while the major one appeared to be a 7-substituted adenine, on the basis of its ultraviolet spectra and pK_a value. Its NMR spectrum showed no vinyl or aldehyde proton absorbtions, but indicated that this adduct contained 2 mol of acrolein to 1 mol of adenine. This product was not examined further.

Reaction of Acrolein with 1-Methyluracil

As reactions of acrolein had been demonstrated for adenine, cytosine, guanine (*7, 9*) derivatives, the reaction of acrolein with 1-methyluracil was also examined. No reaction took place at pH 4.1, and only a trace of product was observed after 7 days at pH 7.0, 25°C. At pH 9.2, in borate buffer, 25°C, about 10% of a new ultraviolet-absorbing substance (R_f 0.63. solvent c) was formed after 5 h. Its uv spectrum indicated a 1,3-dialkyluracil structure. Analysis by NMR indicated that side reaction products of acrolein predominated in the mixture, however. This reaction was not investigated further.

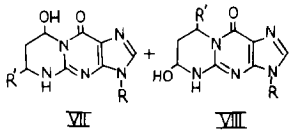
DISCUSSION

Reaction products of acrolein with three of the nucleoside constituents of DNA have now been characterized. All of the structures involve the formation of cyclic

adducts: a new ring has been fused to positions on the bases that normally are involved in Watson-Crick hydrogen bonding. The reactions described are slow, taking many days to proceed to an appreciable extent under physiological conditions. Several nucleic acid components react, however, and the adducts are of a structural type that appears to be significant in mutagenesis and carcinogenesis (*8*). It seems a reasonable presumption, then, that these acrolein reactions have genetic significance. Two sites on a base react to form a cyclic adduct. One is involved in Michael addition across the double bond, and the other condenses with the carbonyl function. Two modes of addition are possible in principle, but only one was observed by us in practice. The amino group added to the acrolein C–C double bond in a Michael addition, and the ring nitrogen combined with the carbonyl group of acrolein. It is not obvious why this orientation predominated, nor does any generalization emerge when we compare these results to related findings.

Chung *et al.* observed products of structures **VIIa** and **VIIIa** (Scheme III) in the reaction of acrolein with deoxyguanosine, though only the former was formed when acrolein was allowed to react with DNA (*7*). The mode of addition illustrated in **VIIb** was the only one noted in the reactions of crotonaldehyde with deoxyguanosine (*18*). On the other hand, both addition modes apparently were observed in the reactions of methyl vinyl ketone (*19*), cyclohexenone (*19*), and *trans*-4-hydroxyl-2-nonenal (*20*) with deoxyguanosine. Finally, some reactions have followed only the path in which the ring nitrogen participates in Michael addition, for example, the reaction of deoxyguanosine with 2-bromoacrolein (*21*) and the noncyclic addition reactions of deoxyadenosine, deoxycytidine, and deoxyguanosine to acrylonitrile (*22*), acrylamide (*23*), and acrylic acid (*24*).

The formation of products **Ib, Ic** and **IVb, IVc** involved the introduction of a new center of assymmetry into molecules that were already chiral. The production of a pair of diastereoisomers might have been expected, but only one product was observed. A similar situation was observed in the formation of **VIIb** in the acrolein–deoxyguanosne reaction (*7*). It is possible that both diastereoisomers were present in these cases but not separated by the methods used, as their properties were similar. Alternatively, it may be that the compounds interconverted too rapidly, via ring-opening, for separation to be achieved. Ring-opening was implied in the oxidation (above) and reduction (*7*) reactions used to characterize **Ia, IVa, VIIa,** and **VIIb.** A ring-opening process was also suggested by the ^1H NMR studies on **Ia** discussed under Results. Diastereoisomers were separated in



VII VIII

a) R = deoxyribosyl, R'=H
b) R = deoxyribosyl, R'=CH_3

SCHEME III

the case of structure **VIIIa** (*7*), though they interconverted with one another upon rechromatography. In this case, the new chiral center was adjacent to an amino-nitrogen, rather than a ring nitrogen. Reduction proceeded with retention of the ring structure rather than ring-opening, and a dehydration pathway was suggested for these processes. Further data are needed to determine whether this observed difference between the two types of cyclic adducts extends more generally.

We examined the reaction of adenine with acrolein in order to test a scheme that assigned a role in prebiotic chemistry acrolein (*5, 17*). It has been proposed that acrolein served as a building block for a hypothetical replicating polymer which proceeded RNA in evolution (*5, 17*). It was suggested that, in serving this function, acrolein would react with purines at the 9-position, and pyrimidines at the 1-position, by simple Michael addition. The replicating polymer would then be formed by further transformations of these monomers and polymerization. Our studies with nucleosides gave no support to this scheme, but rather suggested that acrolein may serve to disrupt genetic functions that rely on Watson-Crick hydrogen bonding. It was possible, however, that unsubstituted bases followed a different reaction path than substituted ones. To test this idea, we examined the reaction of acrolein with adenine. We encountered a cyclic adduct of structure **IVd** and a novel product that contained two molecules of acrolein and appeared to involve 7-substitution on adenine, but no product that fit the requirements of the prebiotic scheme. New data are required for it to be considered further.

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