OXIDATION OF GUANINE AND GUANOSINE BY BROMINE

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Brominating agents, such as bromine and N-bromosuccinimide, have been used extensively for the chemical modification of nucleic acids (Jones and Woodhouse, 1959; Kanngiesser, 1959; Brammer, 1963; Yu and Zamecnik, 1963, 1964; Weil, et. al., 1964; Bakés, et. al., 1965; Duval and Ebel, 1965) and as mutagens (Tsugita and Fraenkel-Conrat, 1962). Three of the common heterocyclic components of ribonucleic acids are known to react with these substances. While the courses of reaction of cytosine and uracil are well known (Duval and Ebel, 1964, and references therein), the reactions of guanine and its derivatives upon bromination have been less clearly established. Several workers have treated guanine and substituted guanines with bromine in organic solvents and obtained the 8-bromo-derivative as the only reported product (Fischer and Reese, 1883; Holmes and Robins, 1964; Duval and Ebel, 1964; Ikehard, et. al., 1965). Other investigators, however, have brominated guanine and its derivatives in dilute aqueous solutions and reported a rapid loss of the ultraviolet absorbance (Suzuki and Ito, 1958; Jones and Woodhouse, 1959; Yu and Zamecnik, 1963; Baev, et. al., 1963). This precludes the production of the 8-bromo-derivative under these conditions as it is strongly ultraviolet-absorbing. We have reinvestigated the reaction of guanine and guanosine with bromine in aqueous solution and have evidence that the reaction proceeds in two stages. The 8-bromo derivate is formed initially. This then is oxidized by bromine with destruction of both rings.

When a suspension of guanine was stirred with excess aqueous bromine at room temperature, it dissolved over the course of several days. After removal of bromine by CCl_4 extraction and aeration, a crystalline compound was recovered, with no melting point below 300°. (Anal. Calcd for $C_3H_5N_3O_3$: C, 27.49; H, 3.84; N, 32.05. Found: C, 27.27; H, 3.94; N, 32.35). It was identified as open-chain oxalyl guanidine: $HO_2CCONHC(NH_2)=NH$. While this compound has been reported previously (Michael, 1894; Schöpf and Kottler, 1939; Wieland and Dekker, 1941; Laursen, et. al., 1957), the properties given by these authors are not in agreement with each other, nor are the compounds described distinguished from the monohydrate of cyclic oxalyl guanidine reported by Traube (1893). Our product, unlike that of Traube, does not lose a mole of water on heating above 160°. It is best characterized by its infrared spectrum, which shows bands in KBr at 2.96, 3.03, 3.21, 3.44, 5.79, 6.02, 6.32, 6.59, 7.00, 7.33, 8.49, 9.03, 9.20, 10.21, 11.44, 12.79, and 14.18 μ, after thorough drying. We have also prepared it by the CrO_3 oxidation of 2-amino-4,6-dihydroxypyrimidine and by the condensation of diethyl oxalate with guanidine carbonate. The latter reaction afforded a methanol soluble product, mp 232-234°, assigned the structure oxalyl diguanidide, $HN=C(NH_2)NHCOCONHC(NH_2)=NH$ (Anal. Calcd for $C_4H_8N_6O_2$: C, 27.89; H, 4.69; N, 48.82. Found: C, 27.96; H, 4.88; N, 49.27), and in addition, an unstable, methanol insoluble, residue. The latter, upon heating with water, gave oxalyl guanidine.

Oxalyl guanidine was produced in 38% yield by bromination of guanine. After removing it by filtration, the following were also isolated from the reaction mixture: oxaluric acid (as ammonium salt) 32%; guanidine (as picrate) 26%; oxalic acid (as calcium salt) 24%. Urea was demonstrated chromatographically by means of p-dimethylaminobenzaldehyde-HCl spray (Hübener, et. al., 1952). It was determined spectrophotometrically by use of the same reagent that urea had been formed in 50% yield. When the reaction with guanine was conducted with a limited amount of bromine,

8-bromoguanine could be detected in the reaction mixture. 8-Bromoguanine itself was found to react with aqueous bromine, with the formation of the same products as in the guanine reaction.

When guanosine was caused to react with excess aqueous bromine, it quickly dissolved and a new product separated out within several minutes. It was recovered by filtration and identified as 8-bromoguanosine by a comparison of its spectral and chromatographic properties with those reported elsewhere (Holmes and Robins, 1964; Duval and Ebel, 1964). It was obtained in 65% yield. This appears to be the most convenient preparative procedure for this substance. If the reaction was allowed to continue without removing the 8-bromoguanosine, this compound reacted further and slowly went into solution. After the removal of bromine and neutralization of the reaction with ammonium bicarbonate, oxalic acid was isolated as its ammonium salt. The remainder of the reaction mixture was examined by paper chromatography and electrophoresis. Guanidine was demonstrated by means of ninhydrin-NaOH spray (Jones and Thompson, 1963) and isolated as its picrate from a portion of the reaction mixture. Only traces of urea were observed, however. Ribose was detected by the use of aniline-phthallate spray (Partridge, 1949) and periodate-benzidine spray (Cifonelli and Smith, 1954). Two other periodate-positive components were detected upon paper electrophoresis in borate buffer at pH 9. One of these, which gave a positive reaction with p-dimethylaminobenzaldehyde-HCl reagent and hydrolyzed in acid to ribose and urea is considered to be a D-ribosylurea (Jones and Walker, 1963). The other periodate-positive component gave a negative reaction to the p-dimethylaminobenzaldehyde-HCl reagent, but gave ribose, oxalate, and urea upon acidic or alkaline hydrolysis. It had considerable mobility as an anion upon paper electrophoresis at pH 7 and is considered to be a D-ribosyloxaluric acid. Its ultraviolet spectrum (H_2O) (λ max = 212 mμ) resembled that of oxaluric acid (λ max = 215 mμ).

In the procedures described above, the rates of the two stages of

reaction (bromination at the 8-position and oxidation) were controlled by the relative solubilities of the starting materials and their 8-bromo-derivatives. When the reaction with guanosine was conducted in dilute aqueous solution, however, at pH values from 1 to 7, the ultraviolet absorbtion was seen to disappear within minutes. Thus, bromination and oxidation occur in rapid succession under these conditions. It seems quite likely then, that one effect of the bromination of nucleic acids is to destroy the guanine residues, degrading them to ribosylurea or ribosyloxaluric acid moieties, or removing them entirely. This would tend to weaken any existing secondary structure by the disruption of guanine-cytosine hydrogen bonds. The destruction of guanine residues may also be responsible, in part, for the mutagenic activity of brominating agents (Tsugita and Fraenkel-Conrat, 1962). Visible light, in the presence of methylene blue, is known to oxidize guanine and has been reported to induce mutations in bacteriophage (Orgel, 1965).

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