ON THE STRUCTURE OF NEOGUANOSINE

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Relatively few purine nucleosides and nucleotides in which the sugar was attached to the purine at other than the 9 - position have been found in nature. The brief list of such compounds includes nucleotides (7-substituted purines) isolated from vitamin B_{12} coenzymes (1), 3-ribosylxanthine (2), and 3-ribosyluric acid (3). It was of interest, therefore, when neoguanylic acid, a new nucleotide isolated from yeast RNA and from commercial guanylic acid, was characterized as a 1-ribosylguanine phosphate (4). However, evidence that the ribosyl-phosphate group was attached at the 1 - position of guanine was derived entirely from a comparison of its ultraviolet spectra in 0.1 N acid and 0.1 N alkali with those of methylated guanines. We have re-examined this assignment of structure, using the corresponding nucleoside, neoguanosine, and wish to present evidence to show that neoguanosine is not a 1 - substituted guanine but is rather N^2 - ribosylguanine.

Neoguanylic acid was isolated from commercial guanylic acid (Nutritional Biochemicals Corporation) by ion exchange chromatography as described by Hemmens (4). Due to the lability of the glycosidic linkage, it was found advisable to remove the compound quickly from the acidic eluate by adsorbtion onto charcoal and subsequent elution with ethanol - conc. NH_4OH - H_2O, 47-3-50. The ultraviolet spectral characteristics of the product agreed with those reported (4). The phosphate ester of neoguanylic acid was cleaved by alkaline phosphatase, as described by Hemmens (4). The product was purified by adsorbtion onto charcoal and elution, and by preparative paper chromatography (to remove traces of guanine and neoguanylic

acid). Finally, it was dissolved in 95% ethanol and filtered, and the filtrate cooled and concentrated. This precipitated neoguanosine as a white solid which decomposed without melting when heated above 180°. Its ultraviolet spectra, in 0.1 N acid and 0.1 N alkali, were similar to those of the nucleotide. The mobilities of neoguanosine upon paper chromatography in three solvent systems were comparable to those in the literature (4). In a fourth solvent system, aqueous ammonium bicarbonate, 85% saturated, we found neoguanosine (R_F 0.65) to run slightly ahead of guanosine (R_F 0.61). This was expected, as neoguanylic acid was reported to run slightly ahead of guanylic acid in the same solvent system (4). The reported R_F of neoguanosine (0.42), however was considerably slower, and comparable to that of that of guanine. We feel that this unusual R_F value may be the result of a misprint or error. The properties of neoguanosine that led us to conclude that it was N^2 - ribosylguanine are summarized below.

The dissociation constants for neoguanosine were determined spectrophotometrically. It was found to have pKa's of 3.3, 8.8, and 12.6. Each value was determined by using buffers of the appropriate pH and taking at least eight points. Spectrophotometric titration of neoguanylic acid was reported to reveal pKa's of 3.1, 9.9, and 12.5 (4). The discrepancy between the middle pK's of the two substances may be due to an intrinsic difference between them. Alternatively, the pKa's of the nucleotide may be less reliable as they were determined on the basis of only three points, and in unbuffered solutions. Our determination of the second pK of the nucleoside was confirmed by electrophoresis in glycine buffer, pH 9.2. Neoguanosine was seen to migrate faster than guanosine (pKa 9.2). More important than the actual values of the pKa's, however, is the fact that there are two dissociations in the alkaline pH region. This is characteristic of guanine, but not of such derivatives as 1-, 7-, or 9 - methylguanine, where one of the potentially acidic ring protons has been replaced by a substituent (5). 1 - methylguanine has pKa values of

3.13 and 10.54 (5). The pKa's of N^2-methylguanine, which had not previously been determined, were found by us to be 3.3, 8.9, and 12.8.

Ultraviolet spectra were taken of neoguanosine as a cation (pH 1.3), neutral species (pH 7), monoanion (pH 11.2), and dianion (pH 14) : $\lambda_{max}^{pH\ 1.3}$ 249, 273 (shoulder) mμ; $\lambda_{min}^{pH\ 1.3}$ 225 mμ; $\lambda_{max}^{pH\ 7}$ 248, 272 mμ; $\lambda_{min}^{pH\ 7}$ 225, 264 mμ; $\lambda_{max}^{pH\ 11.2}$ 249, 271 mμ; $\lambda_{min}^{pH\ 11.2}$ 236, 257 mμ; $\lambda_{max}^{pH\ 14}$ 255 (inflection), 274 mμ; $\lambda_{min}^{pH\ 14}$ 242 mμ. These were quite similar to the corresponding spectra of N^2 - methylguanine as reported in the literature (6,7) and determined by us: $\lambda_{max}^{pH\ 1.3}$ 252, 275 (shoulder) mμ; $\lambda_{min}^{pH\ 1.3}$ 227; $\lambda_{max}^{pH\ 7}$ 249, 277 mμ; $\lambda_{min}^{pH\ 7}$ 227, 266 mμ; $\lambda_{max}^{pH\ 11.2}$ 245, 276 mμ; $\lambda_{min}^{pH\ 11.2}$ 240, 260; $\lambda_{max}^{pH\ 14}$ 255 (inflection), 277 mμ; $\lambda_{min}^{pH\ 14}$ 245 mμ. The spectra of other monomethylguanines were also available in the literature for comparison. The spectra of the cations corresponding to 3-methylguanine (8) and 2-methylamino-6-methoxypurine (9) were quite different from that of neoguanosine. The spectra of 1 -, 7 -, and 9 - methylguanine (5),as monoanions, differed from that of the monoanion of neoguanosine. As already mentioned, the last three methylguanines did not form dianions at pH values up to 14. It should be mentioned that the conclusion to be drawn from these comparisons differs from that derived by Hemmens (4), from a study of ultraviolet spectra in 0.1 N acid and 0.1 N alkali. We feel that his comparison in 0.1 N acid is invalid because his spectral data for N^2-methylguanine ("6-hydroxy-2-methylaminopurine") is incorrect. It disagrees with our own data and with that published elsewhere in the literature (6, 7). It even seems internally inconsistent (it is hard to reconcile a λ -max of 251 with a 250/260 ratio of 0.92). The conclusion drawn from the comparison of spectra in 0.1 alkali also seems invalid. This is because, at that pH, 1-methylguanine exists as a monoanion, neoguanosine largely as a dianion, and N^2-methylguanine as a mixture of both monoanion and dianion.

Guanine and N^2-methylguanine are known to undergo a reaction with glyoxal which can be detected by a characteristic shift in the ultraviolet spectrum (10). The maximum is shifted to shorter wavelengths and there is a decrease in the absorbtion of the shoulder near 270 mμ. 1 - methylguanine, however, shows no change in its spectrum when treated with glyoxal. When neoguanosine was allowed to react with aqueous glyoxal at 37° for 15 hours, there was a shift of the ultraviolet maximum from 249 to 246 mμ, and a decrease in the absorbtion of the minor peak near 270 mμ.

It was reported that the glycosidic bond of neoguanylic acid could be hydrolyzed by heating in 6 N HCl at 100° for 1 hour (4). We have found that neoguanosine can also be hydrolyzed, with the formation of guanine, by heating for 3 hours at 100° in 1.9 N NaOH solution, or even in phosphate buffer at pH 7.3 (3 hours, 100°). This lability under a variety of conditions is not characteristic of nucleosides, or of C-glycosides, but is reminiscent of the behavior of simple aliphatic and aromatic glycosylamines (11).

Neoguanosine was found to resist deamination by nitrous acid, either in 25% acetic acid or in 0.1 N HCl solution. It was not affected by guanase (rabbit liver, Calbiochem.) The infrared spectrum of neoguanosine, taken in KBr, shows a carbonyl band at 5.80 μ, with other bands in this area at 6.23, 6.62, and 6.87 μ.

Identification of neoguanosine as a 1 - substitututed guanine is ruled out by the pK, ultraviolet, and glyoxal reaction data. A 3-substituted guanine structure seems eliminated by ultraviolet spectral data. Neoguanosine also fails to deaminate when heated with alkali, a reaction shown by 3 - methylguanine (12). The infrared and ultraviolet spectral data make a glycosidic link involving the oxygen at the 6 - position unlikely. The pK and ultraviolet spectral data, and the positive reaction of neoguanylic acid with the folin phenol reagent (4) rule out the possibility of 7 - or 9 - substitution. An 8 - ribosylguanine structure does

not explain the ease of hydrolysis of the glycosidic link nor the positive reaction of neoguanylic acid with the Pauly reagent (4). The only formulation for neoguanosine consistant with all of its properties is as N^2 - ribosylguanine. This structure is an unusual one for a nucleoside. Its occurence within intact RNA remains to be demonstrated.

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