tersect any temperature curve. He interpreted this line, or "envelope," as representing the line of maximum density, reasoning that an observed temperature profile cannot cross the line of maximum density. These relationships are shown in Fig. 1. I have also plotted my computed line of maximum density in the same figure. It is significant that temperatures lying to the left of my proposed line of maximum density show colder water overlying warmer water, whereas to the right of the line warmer water overlies colder water. The temperature profiles that are observed to cross the line of maximum density tend to change sign of slope where they cross the line. These observations are consistent with the fact that a stable column of water must have less dense water overlying denser water.

Strøm's scholarly review of the available information about the temperatures of deep lakes is a valuable contribution to the knowledge of lakes. Nevertheless, I believe he erred in identifying his envelope with the line of maximum density. There is no physical reason why the temperature profile of a stable column of water cannot cross the line of maximum density. For instance, in a lake that is mixed vertically until it is isothermal at 3.90°C, the temperature profile will cross the line of maximum density.

HENNING EKLUND
U.S. Public Health Service,
1819 West Pershing Road,
Chicago, Illinois 60609

References

1. S. Wright, *Science* **74**, 413 (1931).
2. N. E. Dorsey, *Properties of Ordinary Water-Substance* (Reinhold, New York, 1940).
3. M. Greenspan and C. E. Tschiegg, *J. Res. Natl. Bur. Std.* **59**, 249 (1957).
4. K. M. Strøm, *Geofys. Publikasjoner* **16**, No. 8 (1945).

3 October 1963

Tautomerism and Protonation of Guanosine

Abstract. *Guanosine has been demonstrated, by infrared and nuclear magnetic resonance spectroscopy, to have a keto-amino structure in neutral aqueous solution and to undergo protonation at N_7 in acid solution.*

Of all the nucleic acid components guanosine (I) has the most complex structure, the largest number of possible tautomeric forms and the widest variety of hydrogen-bonding interactions in which it can or might participate.

From a study of the tautomerism of guanosine we are reporting, without discussing at present the binding of guanosine in nucleic acid, some of our principal conclusions (*1, 2*).

The infrared spectra of D_2O solutions of the compounds were measured with a Beckman IR-7 spectrophotometer with matched cells of 55 μ-path length, (*1, 2*). The spectra (Figs. 1–3) are expressed in terms of frequency in cm^{-1} plotted against absorbance on an arbitrary scale.

The NMR (*3*) spectra were measured in deuterodimethyl sulfoxide or in water; tetramethylsilane (or a water-soluble derivative) was used as internal standard (*4*).

The infrared spectrum of guanosine in D_2O solution (*5*) has strong bands at 1665 cm^{-1}, 1578 cm^{-1}, and ~1568 cm^{-1} (shoulder), a pattern which is quite similar to that of the keto model, 1,9-dimethylguanine (*6*), VI (Figs. 1 and 2). The 1665 cm^{-1} band is assigned primarily to a C_6-carbonyl stretching vibration (probably strongly coupled to the $C_4{=}C_5$ bond but not to the $C_2{=}N_3$ bond) and the others to ring modes. The enol model, 2-amino-6-methoxy-9-β-D-ribofuranosylpurine (*7*), VII, on the other hand, lacks the carbonyl band at 1665 cm^{-1} but has ring vibrations at 1617 cm^{-1} and 1595 cm^{-1} (Fig. 3). We conclude that guanosine has the keto structure I rather than the enol structure II. The spectrum of guanosine in basic solution (Fig. 1) closely resembles that of the enol model, VII, with the ring vibrations shifted to slightly lower frequency in the anion. This correspondence—compare the analogous case of inosine (*2, 8*)—suggests a close similarity in the electronic structures of the enol model (VII) and the anion of guanosine, and, hence, localization of the negative charge to a large extent on the oxygen atom attached to C_6, X.

Upon monoprotonation, guanosine exhibits the spectrum shown in Fig. 1, with bands at 1691 cm^{-1}, 1608 cm^{-1},

Table 1. Infrared spectra in D_2O solution.

Compound	(cm^{-1}) ν_{max}		
Guanosine (I)	1665	1578	1568 (Sh.)
Acid guanosine (V)	1691	1608	1578
Basic guanosine (X)	1628	1591	1576
1,9-Dimethylguanine (VI)	1671	1590	1548
Acid 1,9-dimethyl-guanine	1696	1624	1559.5
Basic 1,9-dimethyl-guanine	1672	1591	1546
VII (enol model)	1617	1594	1527
VIII (7,9-dialkyl model)	1687	1622 1609	1578
Acid VIII (7,9-dialkyl model)	1687.5	1609	1578
Basic VIII (7,9-dialkyl model)	1623	1588.5 1590 (Sh.)	1544
1,7,9-Trimethyl guanine iodide (IX)	1693.5 1708 (Sh.)	1621 1587	1561

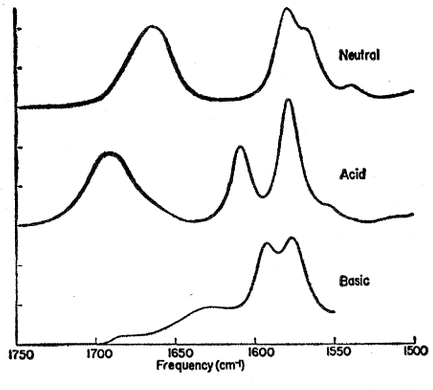


Fig. 1. Infrared spectra of guanosine in neutral ($pD \sim 7$), acid ($pD \sim 1$), and basic ($pD \sim 11$) solutions in D_2O. All infrared spectra are plotted as frequency in cm^{-1} against absorbance on an arbitrary scale.

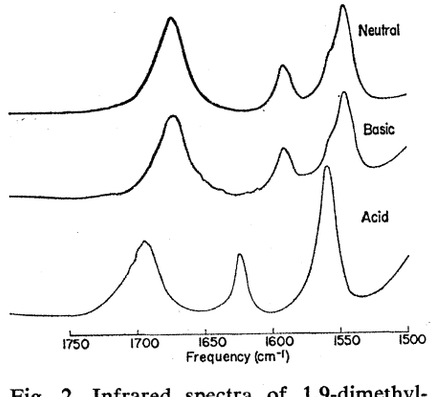


Fig. 2. Infrared spectra of 1,9-dimethylguanine in neutral, basic, and acid D_2O solutions. The similarity of the spectra in basic and in neutral solution results from the lack of an ionizable proton in the model compound.

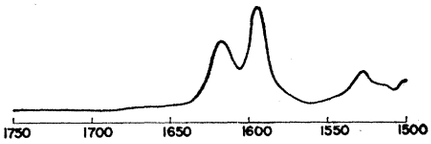


Fig. 3. Infrared spectrum in D_2O solution of 2-amino-6-methoxy-9-β-D-ribofuranosylpurine (VII).

and 1578 cm^{-1}. The presence of the carbonyl band and close similarity to the spectrum of the fixed model compound, 1,7,9-trimethylguanine iodide (IX) (Table 1) indicate a keto structure V for acid guanosine (and lack of protonation on oxygen) and the shift to higher frequency suggests weaker coupling with other multiple bonds than that in the neutral molecule. The other bands are ascribed to ring vibrations. Further, the spectra of the model compounds, 7,9-bis-(2′-hydroxyethyl)-guanine (*9*), VIII, Table 1 (in its cationic form), and 1,7,9-trimethylguanine iodide (*6*) closely resemble that of acid guanosine and so provide clear infrared evidence of the 7-position as the site of protonation, in agreement with an assignment from NMR studies (*10*). The spectrum of the 7,9-dialkyl model (VIII) in a neutral (*p*H 7) solution is merely the sum of the spectra in acid and basic solution, reflecting the change in *p*K of the N_1 proton of about two units caused by 7-alkylation (*9*).

The spectrum of the basic solution of the 7,9-dialkyl model (Table 1) permits us to rule out the possibility of a zwitterionic structure of neutral guanosine (IV) since structure IV

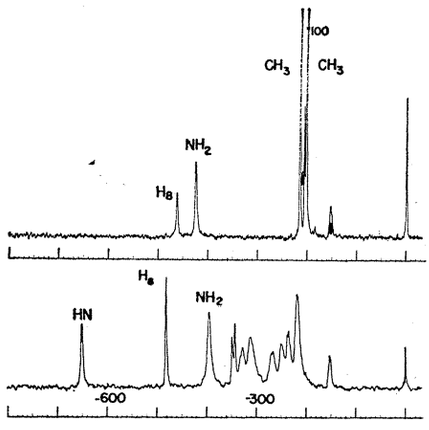
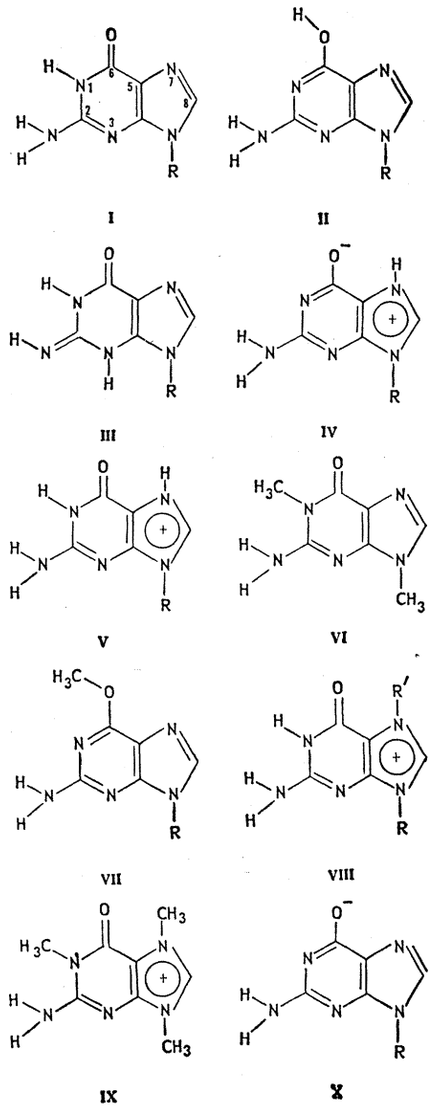


Fig. 4. The NMR spectra in deuterodimethyl sulfoxide of 1,9-dimethylguanine (top) and guanosine (bottom), field increasing from left to right. Scale in cy/sec with TMS as internal reference.



would be isoelectronic with the zwitterion formed from VIII ($R = R' = CH_2CH_2OH$) in basic solution. That is, since neutral guanosine has a spectrum (Fig. 1) greatly different (for example, in the presence of a carbonyl band) from that of VIII, it cannot have the zwitterionic structure IV.

The presence of an amino group can be detected in the infrared by NH_2 stretching vibrations and by an NH_2 bending mode, but neither of these vibrations can be observed in D_2O because of exchange with solvent. We have made a tentative assignment of the NH_2 bending mode of guanosine to a 1639 cm^{-1} band in dimethylsulfoxide (in which the carbonyl band occurs at 1692 cm^{-1}) on the basis of deuteration studies.

We have observed the NMR spectrum of guanosine in dimethylsulf-

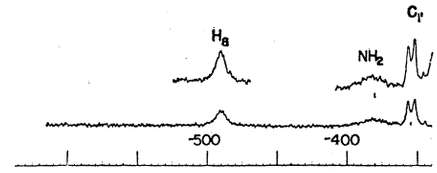


Fig. 5 The NMR spectrum of 5′-GMP in H_2O. Scale as in Fig. 4.

oxide (Fig. 4) and concur in the assignment of other workers (*11*) of the −397 cy/sec band to the NH_2 group, because the imino structure (III) having two nonequivalent protons, would generally be expected to have two separate one-proton peaks. The 1,9-dialkyl model shows an analogous band at −427 cy/sec with an area corresponding to two protons. We have also measured the spectrum of 5′-GMP in H_2O solution (Fig. 5) and have observed bands at −490 cy/sec, −382 cy/sec (broadened by exchange), and a doublet at −354 cy/sec (coupling constant, $J = 5$ cy/sec), which we tentatively assign to the proton at C_8, to the protons of the amino group at C_2, and to the proton attached to the C_1' of the ribose. We have not observed a peak for the proton attached to N_1 in H_2O solution, presumably because of too rapid exchange.

H. Todd Miles
Frank B. Howard
Joe Frazier
Laboratory of Molecular Biology, National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland

References and Notes

1. For tautomeric studies of the other nucleosides and discussion of the infrared spectroscopic methods, see H. T. Miles, *Biochim. Biophys. Acta* **22**, 247 (1956); **27**, 46 (1958); **30**, 324 (1958); **45**, 196 (1960); *Proc. Natl. Acad. Sci. U.S.* **47**, 791 (1961); *J. Am. Chem. Soc.* **85**, 1007 (1963).
2. ———, *Biochim. Biophys. Acta* **35**, 274 (1959).
3. Abbreviations used: NMR, nuclear magnetic resonance; TMA, tetramethylsilane; 5′-GMP, guanosine 5′-phosphate.
4. We thank R. B. Bradley for measuring the NMR spectra.
5. Although infrared spectra of guanosine in neutral aqueous solution have not been previously published, a recent paper [M. Tsuboi, Y. Kyogoku, T. Shimanouchi, *Biochim. Biophys. Acta* **55**, 1 (1962)] reports spectra in acid and in basic D_2O solution with peaks similar to the ones we observe.
6. W. Pfleiderer, *Ann. Chem* **647**, 167 (1961).
7. J. F. Geister, J. W. Jones, R. K. Robins, *J. Org. Chem.* **28**, 945 (1963).
8. H. T. Miles, *Nature* **195**, 459 (1962).
9. P. Brooks and P. D. Lawley, *J. Chem. Soc.* **1961**, 3923 (1961).
10. C. D. Jardetzky and O. Jardetzky, *J. Am. Chem. Soc.* **82**, 222 (1960).
11. J. P. Kokko, J. H. Goldstein, L. Mandell, *ibid.* **83**, 2909 (1961); L. Gatlin and J. C. Davis, Jr., *ibid.* **84**, 4464 (1962).

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