pK'S OF NUCLEIC ACID COMPONENTS AS A FUNCTION OF TEMPERATURE

Chemistry 80 Thesis

by

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pK'S OF NUCLEIC ACID COMPONENTS AS A FUNCTION OF TEMPERATURE

Summary

The pK's of the -N_1H- groups for the several deoxynucleic acid components have been studied as a function of temperature. From this the $\Delta\tilde{H}$'s for the ionizations are obtained. The components studied were thymidine, thymidylic acid, deoxyguanosine, and deoxyguanylic acid (5').

pK's of Nucleic Acid Components as a Function of Temperature

Introduction

A method of investigating the denaturation of DNA is based on considering DNA as a polymer made of monomers having an acid group. This group is important in the hydrogen bonding of the DNA heli··. For such a treatment, it is necessary to know the H of ionization for these acid groups. As an approximation, the H of the monomer acid group can be used for the H for the polymer acid groups. Since the knowledge of pK(T) for the acid group will yield the $\Delta\tilde{H}$, pK(T) was determined for the nucleotides. The work was also done on the nucleosides to determine what differences arise between the two types of compounds. The components studied were thymidine, thymidylic acid, deoxyguanosine, and deoxyguanylic acid (5'). In all four cases the $-N_1H-$ group is the acid group.

The procedure used was the application of pH measurement and titration data to the equation

$$pK_a(T) = pH(T) - \log \frac{(R^{\ominus})}{(RH)} .$$

Experimental

Solutions were made by dissolving deoxyguanosine$\cdot H_2O$ (abbr. guanosine), deoxyguanylic acid (5')$\cdot NH_4$ salt (abbr. guanylic acid),

thymidine, and thymidylic acid·$2H_2O$·$2NH_4$, all A grade, obtained from California Corporation for Biochemical Research, in 0.100 M NaCl. The nucleotides were passed through a 100-200 mesh Bio-Rad AG 50W-X16 cation exchange resin charged with $Na^{\oplus}$ ions. The column was prepared by adding water to 20-30 gm. of resin, then neutraizing with NaOH. Following this, 0.1 M Na Cl was passed through the column until the eluent no longer adsorbed in the UV region. H_2O is passed through the column before the nucleotides. The nucleotide solutions were made with H_2O. After the exchange, they were made up to 0.100 M NaCl. This procedure was used to make the equilibrium more favorable for the exchange of $NH_4^{\oplus}$. Analysis of the final nucleotide solutions was done spectrophotometrically.

The titrant used was 5.00×10^{-3} M NaOH in 0.100 NaCl. All titrations were done with a 5 ml. burette.

To prevent interference of bicarbonate in the titrations, all experiments were carried under CO_2 free conditions. The NaOH solution, made with CO_2 free H_2O, was stored under"Ascarite". Argon was bubbled through all other solutions to drive off the CO_2. After that, all work is done under an argon atmosphere.

All pH measurements were made using a Beckman Model 76 meter, and a Beckman #39170 Fiber Type Calomel reference electrode. In the room temperature nucleotide measurements, a Beckman #41260 Type E-2 glass electrode was used as indicating electrode. In all other experiments, a Beckman #41263 General Purpose glass electrode was used as indicating electrode. All pH measured were below 10.0. With such pH's, according to Beckman Instructions 678-A, the sodium ion

correction is less than 0.02 pH units for both glass electrodes.

For the nucleosides,

$$(R^{\ominus}) = (Na^{\oplus}) - (OH^{\ominus})$$
$$(RH) = C - (R^{\ominus})$$

with $(Na^{\oplus})$ the formal concentration of base added, C the formal concentration of nucleoside, and $(OH^{\ominus})$ the hydroxide concentration calculated from pH data.

Due to the presence of the phosphate group, the nucleotide case is more involved. The phosphate group will be titrated along with the $-N_1H-$. Since pK = 6.5 for the second hydrogen of the phosphate group[1], at pH = 8.5 the singly protonated phosphate concentration is 1/100 that of the unprotonated. This point, $pH_o(T)$, is taken as the starting point for the titration of the $-N_1H-$. For the nucleotides

$$(R^{\ominus}) = \frac{C}{1 + 10^{b}} + (Na^{\oplus}) - \left\{(OH^{\ominus})_f - (OH^{\ominus})_o\right\}$$
$$b = pK_a(T) - pH_o(T)$$
$$(RH) = C - (R^{\ominus})$$

with C the formal concentration of nucleotide, $(Na^{\oplus})$ the formal concentration of base added after the starting point, $(OH^{\ominus})_o$ the hydroxide concentration at the starting point, $(OH^{\ominus})_f$ the hydroxide concentration at the end. The term $C/(1 + 10^{b})$ is a correction for the amount of $-N_1H-$ protons which have been titrated at this starting point, $pH_o(T)$. Since b depends on pK(T), the calculations

are done by successive approximations.

Dilution corrections are made where necessary.

Several alternate procedures were used in obtaining the data. In the first procedure(#1) the temperature of a titrated solution was varied while simultaneously measuring the pH(T). For the second procedure(#2) the solutions were titrated at a fixed temperature while measuring pH. The third procedure(#3) consisted of taking several previously titrated solutions whose pH had been measured at room temperature and measuring their pH at a fixed high temperature (around 45°).

Results and Discussion

Procedure #1 immediately presented problems, when tried with standard buffer. Reproducible results were not obtained with the electrodes in the solution during heating and cooling. This nonreproducibility is explained by the temperature "hysteresis" effect discussed by Mattock[2]. This problem was circumvented by the other procedures which allow standardization of the electrode system and measurement at the same temperature.

The length of time that a titrated solution was used was kept at a minimum as the solutions became more acid with time. This was noticable when the solutions sat for an hour or so. This change was most likely caused by contamination by CO_2 or impurities in the glass.

Table I contains the results of the experiments. Each pK(T) is the results of measurements on a set of titrated samples of the

Table I

Exp. No.	Solution	Procedure	Date	pK(T)	Temp.
1	0.100 M NaCl	2	10/19	13.71	26.0°C
2	"	2	11/8	13.09	49.5
3	"	3	2/11	13.28	43.8
3	"	3	"	13.70	27.7
4	"	3	2/15	13.82	27.0
4	"	3	"	13.31	43.5
5	"	3	5/3	13.76	27.7
5	"	3	"	13.18	43.7
6	1.26×10^{-3} Thym.	2	10/19	9.76	24.0
7	" "	2	11/9	9.31	50.0
8	1.36 "	3	11/29	9.71	24.0
8	" "	3	"	9.23	50.0
9	1.33 "	3	1/28	9.68	23.5
10	" "	3	2/4	9.69	26.0
11	4.19 "	3	2/11	9.67	27.8
11	" "	3	"	9.44	44.0
12	" "	3	2/16	9.67	26.2
12	" "	3	"	9.32	43.2
13	1.35 Thym. acid	3	5/3	9.79	27.7
13	" "	3	"	9.49	44.0
14	1.21 Guan.	3	2/15	9.27	26.8
14	" "	3	"	8.97	43.7
15	" "	3	3/4	9.20	26.5
15	" "	3	"	8.90	43.8
16	.896 Guan. acid	3	5/3	9.66	27.7
16	" "	3	"	9.20	43.5

given stock solution. Values for the $pH_o(T)$, the starting point for the nucleotide titrations, were obtained by using procedure #3 on solutions for which $pH(25^o) = pH_o(25^o)$.

The plot of pK(T) should be linear (Fig.1,2,3). The inconsistences shown here(Fig.1,2,3) are an indication of the poor reproducibility of the experiments. The lines were drawn to represent an approximate best fit.

The indexing of the values point out that values obtained on the same day bear the same relation to other values. This may indicate that something is happening to the stock solutions.

Fig.1 shows that the experimental results for the ionization constant of water in 0.100 M NaCl are slightly lower than the theoretica values. This is probably due to both experimental error and the approximate nature of the Debye-Hückel theory. The "best fit" line yields the values $pK(43.7^o) = 13.24 \pm 0.07$ and $pK(27.0^o) = 13.75 \pm 0.07$. This yields a $\Delta\tilde{H}$ of 13.4 ± 0.4 Kcal. This compares favorably with the $\Delta\tilde{H}$ from the theoretical value ($\Delta\tilde{H} = 13.6$ Kcal.). Thus, we are incouraged to believe that the experimental pK's of this investigation are of satisfactory accuracy and may be used to reduce the data for the nucleoside and nucleotide experiments.

The important final results of this investigation are displayed in Table II.

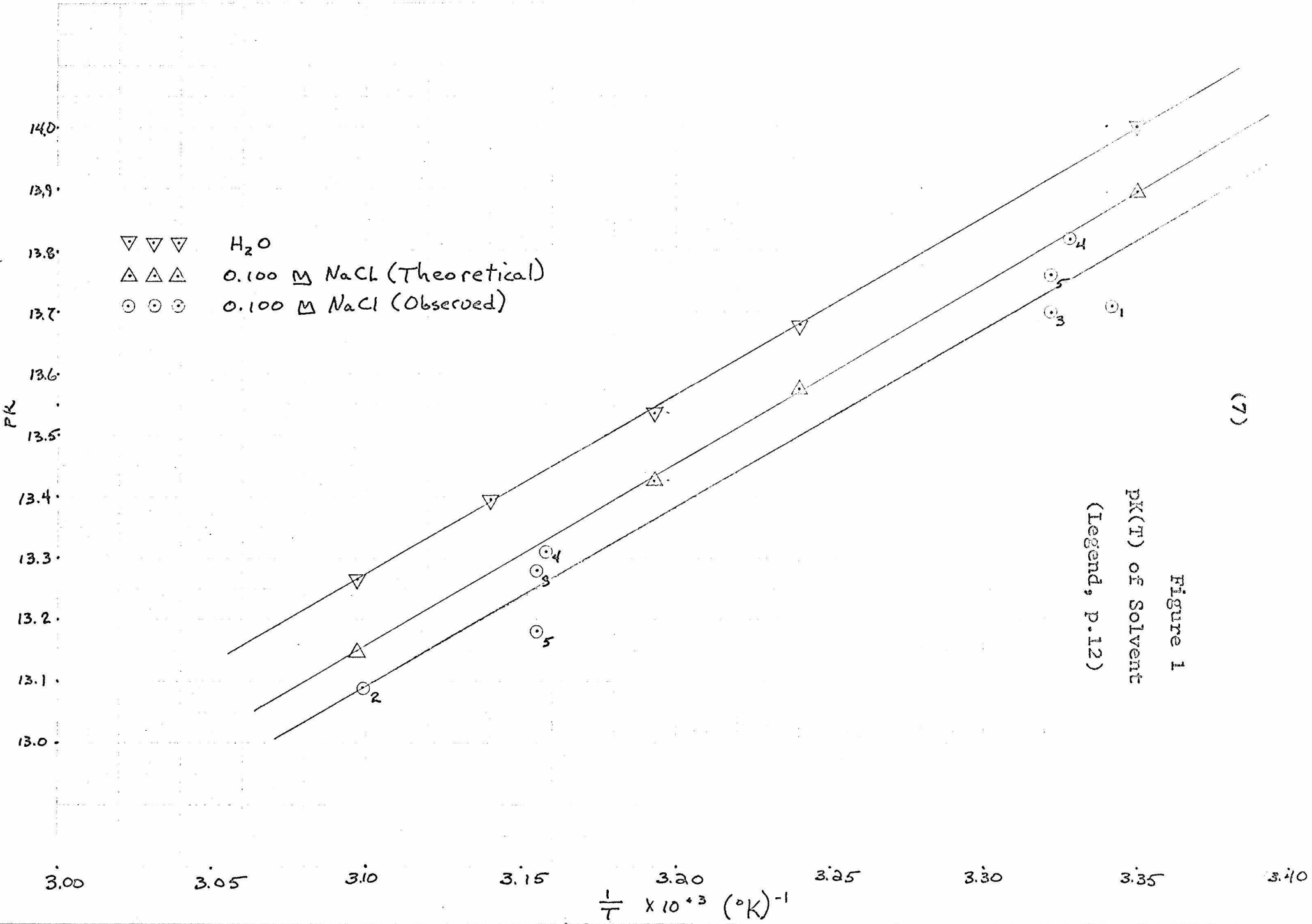

Figure 1

pK(T) of Solvent

(Legend, p.12)

Figure 2

pK(T) of Thymidine and Thymidylic Acid

(Legend, p. 12)

Figure 3

pK(T) of Guanosine and Guanylic Acid

(Legend, p. 12)

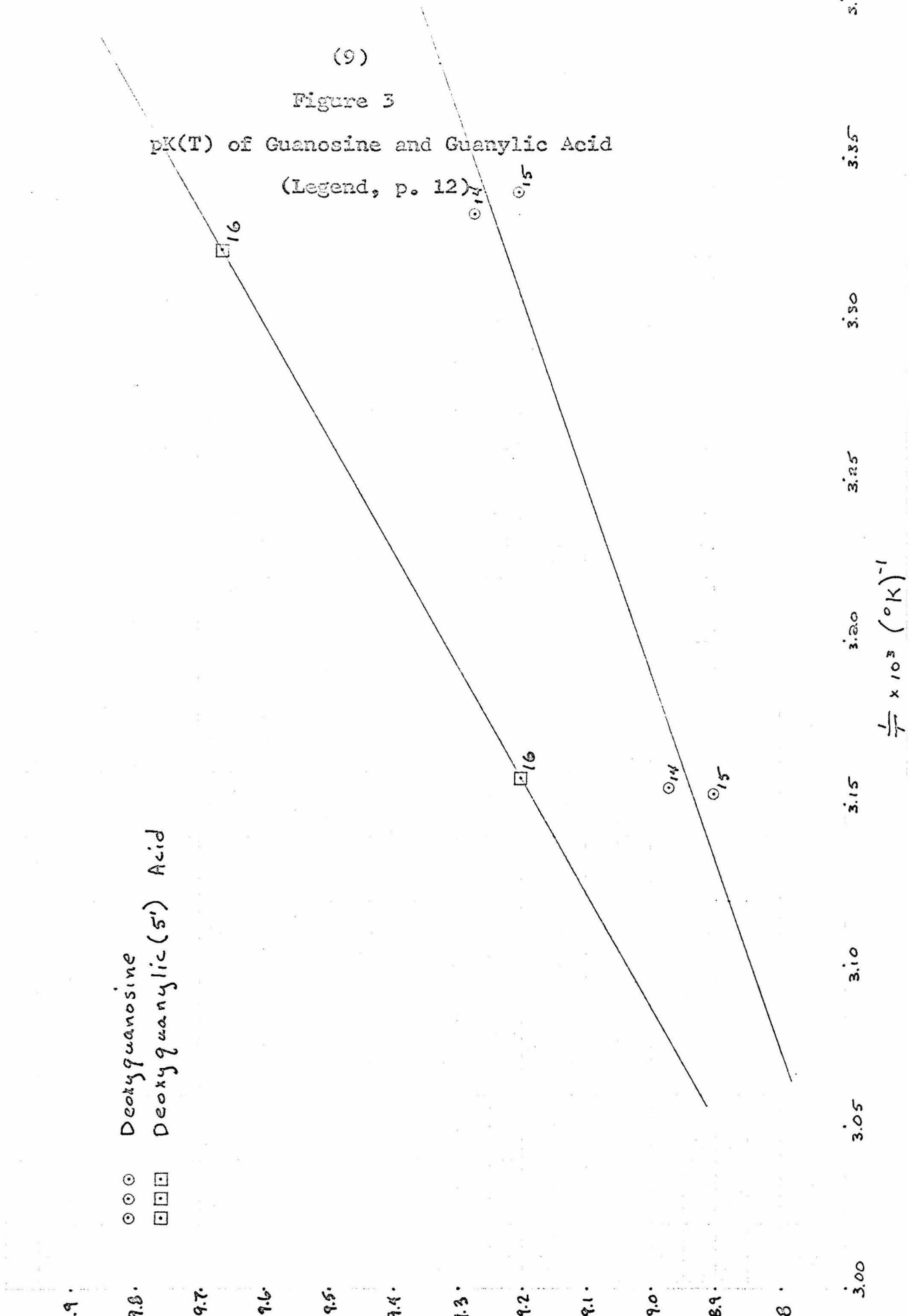

Table II

Compound	pK(25°)	pK(50°)	$\Delta\tilde{H}$
Thymidine	9.69	9.28	7.4 Kcal.
Thymidylic Acid	9.84	9.39	8.1
Guanosine	9.27	8.84	7.7
Guanylic Acid	9.71	9.02	12.4

For comparison, the only values in the literature are 10.0[3] for thymidylic acid at room temperature and 9.7[3] for guanylic acid at room temperature. However these measurements are for solutions of the nucleotides in H_2O. This difference in ionic strength could explain part of the discrepancy in the thymidylic acid case. However there is a lack of a similar discrepancy in the case of guanylic acid. Also to be considered is the fact that the temperature was not reported by the article. Also the precision here is probably better. Either of these latter two points could be used to relate the two sets of data.

According to Table II, thymidine is a slightly weaker acid than guanosine, and in both cases, $\Delta\tilde{H} \cong 7.5$ Kcal.

If we consider the effect of going from the nucleoside to the nucleotide, there is a very slight acid-weakening effect (0.10-0.15 pH units) for the thymine series, with a slight increase in H. However in the guanine series, there is a large change in pK(25) (0.44 pH units) but a smaller change in pK(50) (0.18 pH units),

with a corresponding increase in $\Delta\tilde{H}$ to 12.4 Kcal. It should be noted that the nucleotide measurements were done only once. They are more likely to be in error than the nucleoside results. The electrostatic effect of the doubly negative phosphate group would be acid weakening, in agreement with all observations. It is surprising, however, if this is the only effect, that there should be such a difference between the effects in the thymine and guanine series.

There appear to be four reasonable possibilities.

1) The measurements are all good. There is a much larger effect of the 5' phosphate group in the guanine series only. This would suggest that the 5' phosphate group is hydrogen bonded to the $-N_1H-$, thereby decreasing K_a and increasing $\Delta\tilde{H}$. For some structural reason, this does not occur in thymidylic acid.

2) The guanylic results are wrong and the thymine series typical of all nucleoside-nucleotide changes.

3) The thymidylic results are wrong and the guanine series are typical.

4) The guanine results are not straight forward because of the tendency of guanine compounds to polymerize.[4]

Further careful experiments and checks should make it possible to discriminate between these possibilities.

Legend

Fig. 1 pK(T) of Solvent

The values for the pure water are from the Handbook[5]. The theoretical values for the 0.100 M NaCl were calculated from the Debye-Hückel Extended Limiting Law,

$$pK_S(t) = pK_W + \frac{Z^2(1.8246 \times 10^6)(\epsilon T)^{-3/2} I_v^{1/2}}{1 + (50.29)a(\epsilon T)^{-1/2} I_v^{1/2}}$$

with I_v the volume molar ionic strength, ϵ the dielectric constant of water, a the ionic radius in angstroms, Z the valence of the ion, $pK_W(T)$ the pK of H_2O, $pK_S(T)$ the pK of solution of ionic strength I_v.

Fig. 2 pK(T) of Thymidine and Thymidylic Acid

Fig. 3 pK(T) of Guanosine and Guanylic Acid

Common to all Fig.

The indices on the points of the plots are the Experiment Number (see Table I). These allow easy identification of the results from the same day.

Prospectus

Further investigation, both at the same and different temperatures, is called for in all four cases. Attention should be directed to determining which of the four possibilities is the actual one.

In addition, investigation may be extended to other series of nucleosides and nucleotides.

References

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3.) Gellert, M., Lipsel,M.N., Davies,D.R., Proc. nat. Acad. Sci., Wash. 1962 48 p. 2013.

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5.) Handbook of Chemistry and Physics., Thirtyninth Edition, Cleveland: Chemical Rubber Publishing Co., 1957, page 1643.