277. *Reactions of Nucleic Acids and Their Components. Part II.* Thermodynamic Constants of Adenine.*

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Ionisation constants and other thermodynamic constants of adenine have been evaluated by constant-volume techniques at various temperatures.

For the basic dissociation constant of adenine at 25°, Ogston[1] recorded 4·1, Taylor[2] 4·15, and Alberty, Smith, and Bock[3] 4·12 (in 0·15M-sodium chloride). Albert[4] obtained 4·22 at 20°. The acid dissociation constant has been determined at 25° by Taylor[2] and by Alberty, Smith, and Bock[3] as 9·75 (in 0·15M-sodium chloride).

In the present investigation the ionisation constants of adenine were determined by using a series of solutions containing the same adenine concentration but increasing quantities of acid or base, at 20°, 30°, and 40°, by means of pH measurements at a glass electrode. The entire titration range of adenine was covered. Jordan and Taylor's[5]

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* Part I, preceding paper.

[1] Ogston, *J.*, 1936, 1713.

[2] Taylor, *J.*, 1948, 765.

[3] Alberty, Smith, and Bock, *J. Biol. Chem.*, 1951, **193**, 425.

[4] Albert, *Biochem. J.*, 1953, **54**, 646.

[5] Jordan and Taylor, *J.*, 1946, 994.

water corrections were applied at pH values above 10 and below 4. The ionic strengths at the midpoints of neutralisation on the acid range and the alkaline range, respectively, were calculated to be $I = 0{\cdot}006$ and 0·008 for 0·0025M-adenine and 0·009 and 0·012 for 0·00375M-adenine. Owing to the uncertainty concerning the activity coefficient values of adenine, the ionic strengths computed are considered accurate only to the third decimal place. The pH values were checked several times during 48 hours and reproducibility was better than 0·01 pH unit. The results are given in Table 1.

TABLE 1.

Ionisation constants of adenine at various temperatures.

Temp.	20°	30°	40°
pK (NH_2)	4·22	4·12	4·06
pK (NH)	9·96	9·67	9·49

Controls with 0·001M-adenine gave identical results. Owing to the very low buffering capacity at lower concentrations, it was impracticable to determine the pK values at lower ionic strengths. Alberty, Smith, and Bock's data [3] which were evaluated in 0·15M-sodium chloride, *i.e.*, at ionic strength values thirteen times as great as the highest values involved in this investigation, are only 0·05 pH unit below those recorded by us.

In view of the practically identical results of this investigation and those of Taylor [2] and Albert,[4] it seems reasonable to assume that the present results should have practically the same values as those at zero ionic strength and on this basis to calculate the approximate $\Delta H°$, $\Delta G°$, and $\Delta S°$ ionisation values. These are given in Table 2.

TABLE 2.

Thermodynamic constants of adenine at various temperatures (ΔG and ΔH in kcal. mole^{-1}; ΔS in cal. deg.$^{-1}$ mole^{-1}).

Temp.	ΔG (NH_2)	ΔG (NH)	ΔH (NH_2)	ΔH (NH)	ΔS (NH_2)	ΔS (NH)
20°	5·66	13·36				
25			3·8	11·0	-5 ± 2	-5 ± 2
30	5·72	13·43				
35			2·7	8·2	-10 ± 4	-14 ± 6
40	5·83	13·61				

$\Delta G \pm 0{\cdot}04$. $\Delta H \pm 0{\cdot}2$.

Experimental.—Adenine sulphate (British Drug Houses) and adenine (California Foundation for Biochemical Research) were used. Doubly distilled water, or deionised water, free from carbon dioxide, was used. Solutions were kept under anaerobic conditions, deaeration having been achieved by bubbling nitrogen free from carbon dioxide and oxygen through the solutions. Sodium hydroxide solutions were carbonate-free. Toluene was added for bacteriostatic purposes. Temperature control was within ±0·05°. Measurements were carried out in thermojacketed vessels.[6] Calibrations of the glass electrodes were based on values given by the National Bureau of Standards [7] by using 0·05M-potassium hydrogen phthalate and 0·05M-borate.[8]

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[6] Lewin, *Chem. and Ind.*, 1953, 1193.
[7] Nat. Bur. Standards, U.S., Letter Circular 993, August 19th, 1950.
[8] Lewin, *Lab. Practice*, 1955, 441.