Thermodynamic Quantities for the Dissociation Equilibria of Biologically Important Compounds

5. THE SECOND ACID DISSOCIATION OF 2-AMINOETHANOL 1-PHOSPHORIC ACID

By H. B. CLARKE, S. P. DATTA and B. R. RABIN
Department of Biochemistry, University College, London

(*Received* 25 *August* 1954)

2-Aminoethanol 1-phosphoric acid is widely distributed in animal tissues both in the free state (Walker, 1952) and in combination (Campbell & Work, 1952); it may be synthesized easily (Outhouse, 1937) and thus lends itself to study as a model for other compounds containing both ester phosphate and amino groups, e.g. creatine phosphate.

THEORY

From the pH titration curves of 2-aminoethanol 1-phosphoric acid with NaOH and HCl, and from the formol titration (Figs. 1 and 2) it is seen that the three acid dissociations are widely separated and that the dissociation near pH 10 may be attributed to the amino group. From these considerations, and by analogy with taurine (2-aminoethanol 1-sulphonic acid; King, 1953), the three acid-base equilibria may be represented as shown below, the free ester being a dipolar ion (II).

It will be convenient to refer to these various species as R^+, R^{+-}, R^{+2-} and R^{2-} respectively.

K_{2a} has been determined on the molal scale in cells without liquid junction of the type:

Pt, H_2 (1 atm.) | 2-aminoethanol 1-phosphoric acid (m_1), NaOH (m_2), NaCl (m_3) | AgCl, Ag

at 5° intervals from 5 to 50°.

The e.m.f. of this cell is given by

$$E = E_0 - \frac{RT}{F} \ln a_{H^+} . a_{Cl^-} . \qquad (1)$$

From this equation, in terms of molalities, and using the Guntelberg approximation for the activity coefficients (Ashby, Crook & Datta, 1954*a*) we get

$$-\log m_{H^+} = \frac{(E-E_0)F}{2{\cdot}3026RT} + \log m_{Cl^-} - \frac{2AI^{\frac{1}{2}}}{1+I^{\frac{1}{2}}} \qquad (2)$$

the linear term in I being neglected.

The dissociation constant under consideration is

$$K_{2a} = \frac{a_{H^+} . a_{R^{+2-}}}{a_{R^{+-}}} . \qquad (3)$$

Combining eqns. 1 and 3 and eliminating m_{H^+} we get

$$-\log K_{2a} = \frac{(E-E_0)F}{2{\cdot}3026RT} + \log \frac{m_{Cl^-} . m_{R^{+-}}}{m_{R^{+2-}}} + \log \frac{\gamma_{Cl^-} . \gamma_{R^{+-}}}{\gamma_{R^{+2-}}} . \qquad (4)$$

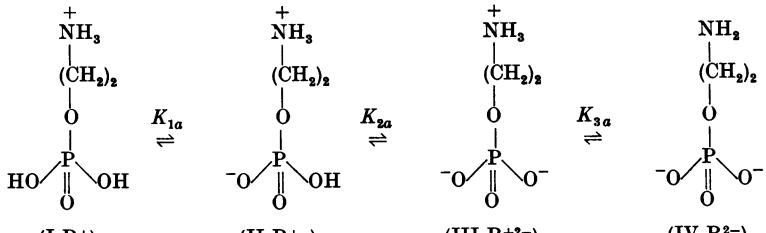
The dipolar ion R^{+-} may be considered as an uncharged molecule with unit activity coefficient, and the ion R^{+2-} may be considered as carrying a unit negative charge. Thus the activity coefficient term in eqn. 4, when approximated as before, reduces to a linear term in I, and eqn. 4 becomes

$$-\log K_{2a} + \beta I = \frac{(E-E_0)F}{2{\cdot}3026RT} + \log \frac{m_{Cl^-} . m_{R^{+-}}}{m_{R^{+2-}}} = y, \qquad (5)$$

where $m_{R^{+-}} = m_1 - m_2 - m_{H^+}$, $m_{R^{+2-}} = m_2 + m_{H^+}$ and $m_{Cl^-} = m_3$; and

$$I = m_2 + m_3 + m_{H^+} . \qquad (6)$$

In the solutions studied m_{H^+} was so small that the value at one temperature only was evaluated and used in all calculations.



(I R^+) (II R^{+-}) (III R^{+2-}) (IV R^{2-})

The right-hand side (y) of eqn. 5 is plotted against I to give a straight line of slope 'β'; the intercept at $I=0$, giving $-\log K_{2a}$, is obtained by the method of least squares.

The values of $-\log K_{2a}$ at different temperatures were found to fit the Harned & Robinson (1940) equation

$$-\log K_{2a} = A/T - D + CT. \qquad (7)$$

The constants of this equation were calculated for each set of dilutions and their standard deviations calculated by the methods previously described (Please, 1954).

The values of pK_{1a}, pK_{2a} and pK_{3a} on the molar scale were estimated by pH titration curve measurement, with a glass electrode at 20°. They were obtained from the titration curves by the well-known equation

$$pK = pH + \log \frac{a_{\text{acid species}}}{a_{\text{base species}}}.$$

This equation, in terms of molarities and with the same approximation for the activity coefficients, becomes for K_{1a}, K_{2a} and K_{3a} respectively:

$$pK_{1a} = pH + \log \frac{C_{R+}}{C_{R+-}} - \frac{AI^{\frac{1}{2}}}{1+I^{\frac{1}{2}}} - \beta_1 I, \qquad (8)$$

$$pK_{2a} = pH + \log \frac{C_{R+-}}{C_{R^{+2}-}} + \frac{AI^{\frac{1}{2}}}{1+I^{\frac{1}{2}}} - \beta_2 I, \qquad (9)$$

$$pK_{3a} = pH + \log \frac{C_{R^{+2}-}}{C_{R^{2}-}} + \frac{3AI^{\frac{1}{2}}}{1+I^{\frac{1}{2}}} - \beta_3 I. \qquad (10)$$

The values of the coefficients β_1, β_2 and β_3 cannot be determined from a single titration curve, and as they are usually small they may be neglected to a first approximation.

Fundamental constants. The values of E_0 for the silver–silver chloride electrode of Harned & Ehlers (1933) were used as before (Ashby *et al.* 1954*a*) but

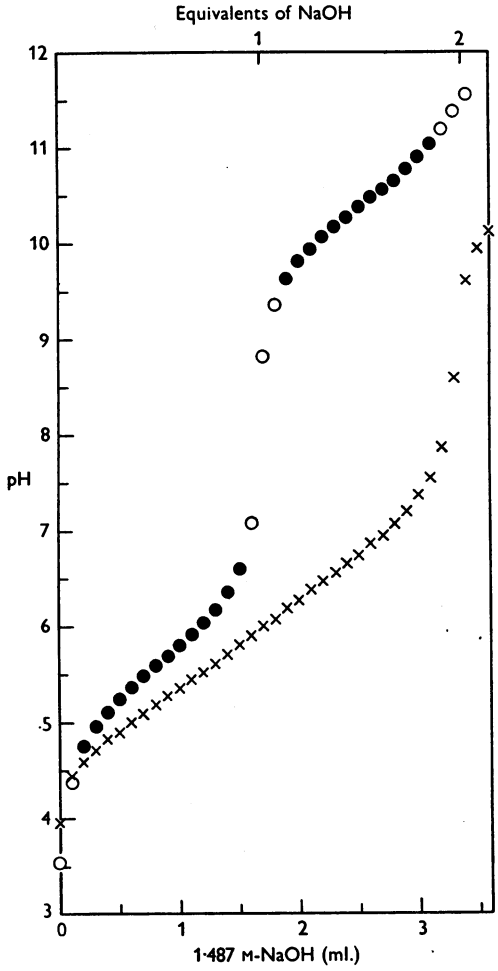


Fig. 1. Alkaline pH titration curve of 2-aminoethanol 1-phosphoric acid with and without formaldehyde, 20°. ○○●●, 25 ml. 0·1 M 2-aminoethanol 1-phosphoric acid +25 ml. 0·3 M-NaCl; × × × ×, 25 ml. 0·1 M 2-aminoethanol 1-phosphoric acid + 15 ml. 40% (w/v) formaldehyde + 10 ml. water. The full points were used to calculate pK_{2a} and pK_{3a}.

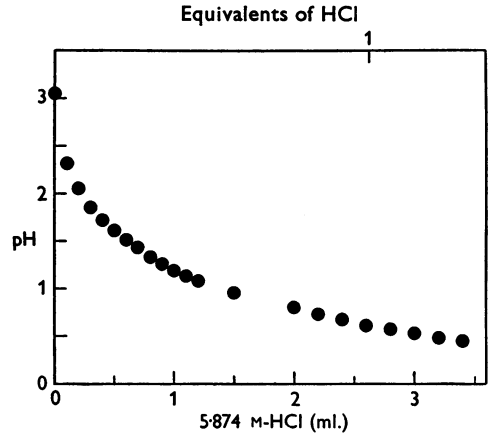


Fig. 2. Acid pH titration curve of 2-aminoethanol 1-phosphoric acid, 20°. ●●●●, 2·1723 g. 2-aminoethanol 1-phosphoric acid in 20 ml. water.

Table 1. *Values of fundamental constants used in this work*

$R = 8{\cdot}31436$ joules (absolute) deg.$^{-1}$ mol.$^{-1}$ (chemical scale).

$T = 273{\cdot}16 + t°$ C.

$F = 96493{\cdot}9$ coulombs (absolute) g.mol.$^{-1}$ (chemical scale).

1 cal. (defined) = 4·1840 joules (absolute).

The values of R and F are from the values given by DuMond & Cohen (1953) on the physical scale divided by 1·000272. The values of T and the ratio J/cal. are those given by Rossini, Gucker, Johnston, Pauling & Vinal (1952).

were converted into absolute volts by multiplying by the factor 1·00033 (DuMond & Cohen, 1948). All e.m.f. measurements and calculations were done in absolute volts. The most recent values of other constants were used; these are listed in Table 1.

METHODS

The apparatus and general technique for the measurements in cells without liquid junction have been described previously (Ashby *et al.* 1954*a*). The measurement of temperature, the preparation of carbonate-free NaOH and standard HCl, the glass electrode standardization and measurements have also been described (Clarke, Cusworth & Datta, 1954).

Preparation of 2-aminoethanol 1-phosphoric acid. The ester was prepared by allowing $POCl_3$ to react with aqueous 2-aminoethanol as described by Outhouse (1937). The reaction mixture was diluted with *ca.* 20 vol. water and passed through columns of 'Deacidite FF' (The Permutit Co. Ltd., London, W. 4), a strongly basic anion-exchange resin, in the hydroxyl phase. The columns were then thoroughly washed with boiled-out distilled water to remove free 2-aminoethanol. The 2-aminoethanol 1-phosphoric acid was then displaced from the resin with M-HCl. The ester came off first, followed by phosphoric acid and HCl. The pH of the liquid containing the ester was between 4 and 2. Fractions were tested for reaction with ninhydrin, for inorganic and ester phosphate with acid molybdate, and for chloride. Those fractions giving both positive reactions with ninhydrin and for bound phosphate, but no reaction for inorganic phosphate or chloride, were combined and evaporated to dryness under reduced pressure. The residue, which was slightly coloured, was dissolved in a small amount of distilled water, boiled with decolorizing charcoal and filtered. The clear filtrate was then treated with 4 vol. methanol. The free ester crystallized almost immediately, and more came out of solution on standing overnight in the refrigerator. The crystals were filtered off, washed with methanol and dried *in vacuo* over P_2O_5. The ester was twice reprecipitated with methanol from water and finally recrystallized from conductivity water.

Typical analytical results were as follows: m.p. (corr.) 243·5–244·5°; 243·0–244·0° (Outhouse, 1937, 244°); inorganic P: 0·04 and 0·00% of total P; total P: 99·73 and 100·40% of required amount; total N: 99·3, 99·7, 99·97, 99·90 and 100·1% of required amount. Chromatography showed only one ninhydrin-reacting spot.

The ester was considered to be highly purified 2-aminoethanol 1-phosphoric acid. There was no water of crystallization.

Sodium chloride (AnalaR) was recrystallized once from water with HCl gas and ignited. No free acid could be detected in the salt.

Formaldehyde for the formol titration was shaken with heavy magnesium carbonate and the titration was carried out according to the method of Dunn & Loshakoff (1936).

RESULTS

The molalities and ionic strengths of the solutions, the e.m.f.'s of the cells and the extrapolation function y (eqn. 5) of the solutions examined in cells without liquid junction are shown in Table 2. The extrapolated values of $-\log K_{2a}$ (pK_{2a} (obs.)) at infinite dilution, the slopes 'β' of the extrapolations and the values of the ratio $m_{R^+-}/m_{R^{+\pm}-}$ at 25° are given in Table 3. The values of pK_{2a} (calc.) were derived from eqn. 7, the constants of which were obtained by averaging the coefficients of the orthogonal polynomials used in fitting the curves relating pK_{2a} (obs.) to temperature for each set of results. There was no significant difference between the three sets. The values of the constants of eqn. 7 and the minimum value of pK_{2a} together with the temperature at which it occurs are also given. The thermodynamic quantities associated with the second acid dissociation are given in Table 4.

The mean values of pK_{1a}, pK_{2a} and pK_{3a} on the molar scale, obtained from points on the pH titration curves (Figs. 1 and 2) and eqns. 8–10, neglecting the linear term in I, are given in Table 5. The number of points on the pH titration curves used in the calculations, together with the standard errors, are also given.

The value of pK_{1a} is given as <1 because the values drifted downwards from 0·68 to 0·30 as the titration with HCl proceeded. This pK may be considered so low as to be inaccessible to measurement by this method with a glass electrode. The solutions must of necessity be strongly acid, and the corrections in the molarity terms due to the hydrogen-ion concentration must be large.

For pK_{2a}, values for β_2 are available, being very nearly the same as for 'β' in eqn. 5; the difference between the molar and the molal scales is negligible at 20°. Values of 'β_2' for the appropriate buffer ratios $m_{R^+-}/m_{R^{+\pm}-}$ in the titration curve were found from Fig. 3. The mean value of pK_{2a} thus corrected is also given in Table 5; it may be seen that the standard deviation is slightly reduced by this correction. For comparison the value of pK_{2a} at 20° from cells without liquid junction, converted to the molar scale by applying the correction given in Ashby *et al.* (1954*a*; Table 2, p. 195), is also given. The corrected value of pK_{2a} from the pH titration curve is seen to be very close to that obtained from cells without liquid junction, the difference being within the tolerance of both the pH scale and the primary standard of pH (British Standards Institution (1950) 1647).

In Table 6 are collected the pK values of 2-aminoethanol 1-phosphoric acid, some other phosphoric acid esters, orthophosphoric acid, taurine and 2-aminoethanol.

DISCUSSION

The results given in Table 3 show that the assumptions about the activity coefficients of the various ionic species and their contributions to the ionic strength are justified. If these assumptions were seriously incorrect, the values of pK_{2a} (obs.)

Table 2. *2-Aminoethanol 1-phosphoric acid. Molalities (m) and ionic strengths (I) of solutions, e.m.f.'s of cells (E), absolute volts (corrected to 1 atm. pressure of hydrogen and rounded temperatures) and extrapolation functions (y)*

m_1 = molality of 2-aminoethanol 1-phosphoric acid; m_2 = molality NaOH; m_3 = molality NaCl; m_{H^+} = antilog $[-(E-E_0)\ F/2{\cdot}3026RT - \log m_{Cl^-} + 2AI^{\frac{1}{2}}/(1+I^{\frac{1}{2}})]$; $I = m_2 + m_3 + m_{H^+}$; $y = -\log K_{2a} + \beta I = (E-E_0)\ F/2{\cdot}3026RT + \log m_{Cl^-} . m_{R^+-}/m_{R^{+\pm}-}$.

Set 1

$10^2 m_1$		0·79277	1·98223	3·17152	4·36069	5·54959	6·73850	7·92719	9·08142
$10^2 m_2$		0·39979	0·99963	1·59938	2·19906	2·79863	3·39819	3·99764	4·57971
$10^2 m_3$		0·39988	0·99986	1·59976	2·19958	2·79928	3·39899	3·99857	4·58078
$10^6 m_{H^+}$	(25°)	2	2	2	2	2	2	3	3
$10^2 I$		0·79985	1·99969	3·19935	4·39886	5·59814	6·79742	7·99646	9·16075
5°	*E*	0·68867	0·66637	0·65501	0·64707	0·64109	0·63629	0·63226	0·62880
	y	5·83276	5·82689	5·82522	5·81966	5·81602	5·81338	5·81090	5·80724
10°	*E*	0·69393	0·67128	0·65968	0·65161	0·64554	0·64068	0·63659	0·63306
	y	5·82824	5·82328	5·82097	5·81564	5·81231	5·81013	5·80788	5·80408
15°	*E*	0·69924	0·67618	0·66439	0·65620	0·65001	0·64505	0·64089	0·63731
	y	5·82707	5·82193	5·81989	5·81494	5·81139	5·80897	5·80675	5·80316
20°	*E*	0·70454	0·68112	0·66909	0·66078	0·65448	0·64948	0·64524	0·64161
	y	5·82868	5·82423	5·82158	5·81701	5·81342	5·81178	5·80943	5·80605
25°	*E*	0·70991	0·68604	0·67383	0·66538	0·65900	0·65386	0·64955	0·64584
	y	5·83407	5·82877	5·82654	5·82201	5·81888	5·81632	5·81401	5·81032
30°	*E*	0·71523	0·69095	0·67854	0·66994	0·66348	0·65824	0·65386	0·65007
	y	5·84115	5·83569	5·83354	5·82887	5·82619	5·82340	5·82113	5·81714
35°	*E*	0·72047	0·69585	0·68317	0·67445	0·66791	0·66260	0·65812	0·65434
	y	5·85016	5·84568	5·84247	5·83815	5·83590	5·83338	5·83066	5·82786
40°	*E*	0·72583	0·70071	0·68787	0·67900	0·67231	0·66693	0·66232	0·65853
	y	5·86301	5·85691	5·85443	5·84998	5·84703	5·84477	5·84112	5·83915
45°	*E*	0·73113	0·70558	0·69245	0·68352	0·67655	0·67128	0·66652	0·66270
	y	5·87708	5·87053	5·86670	5·86354	5·85784	5·85869	5·85383	5·85234
50°	*E*	0·73633	0·71041	0·69690	0·68801	0·68097	0·67564	0·67072	0·66669
	y	5·88993	5·88386	5·87733	5·87698	5·87190	5·87310	5·86691	5·86309

Set 2

$10^2 m_1$		0·90217	1·80448	2·70681	3·60914	4·51145	5·41375	6·31613	7·21843
$10^2 m_2$		0·57349	1·14707	1·72066	2·29426	2·86784	3·44141	4·01503	4·58861
$10^2 m_3$		0·62629	1·25268	1·87908	2·50549	3·13187	3·75825	4·38469	5·01107
$10^6 m_{H^+}$	(35°)	1	1	1	1	1·5	2	2	2
$10^2 I$		1·19989	2·39987	3·59987	4·79989	5·99986	7·19982	8·39989	9·59986
5°	*E*	0·69118	0·67418	0·66472	0·65767	0·65209	0·64758	0·64376	0·64054
	y	5·83894	5·83206	5·83679	5·83398	5·82980	5·82728	5·82502	5·82468
10°	*E*	0·69652	0·67923	0·66957	0·66238	0·65674	0·65215	0·64825	0·64499
	y	5·83504	5·82844	5·83264	5·82960	5·82613	5·82363	5·82117	5·82114
15°	*E*	0·70187	0·68429	0·67446	0·66711	0·66139	0·65671	0·65277	0·64943
	y	5·83377	5·82744	5·83165	5·82802	5·82490	5·82225	5·82029	5·81987
20°	*E*	0·70724	0·68940	0·67933	0·67186	0·66608	0·66131	0·65732	0·65391
	y	5·83580	5·83024	5·83325	5·82976	5·82731	5·82450	5·82286	5·82224
25°	*E*	0·71263	0·69449	0·68421	0·67658	0·67075	0·66590	0·66185	0·65838
	y	5·84075	5·83526	5·83763	5·83359	5·83196	5·82918	5·82767	5·82701
30°	*E*	0·71798	0·69955	0·68902	0·68125	0·67538	0·67042	0·66633	0·66282
	y	5·84757	5·84232	5·84340	5·83916	5·83849	5·83523	5·83419	5·83383
35°	*E*	0·72336	0·70461	0·69383	0·68591	0·68007	0·67498	0·67082	0·66727
	y	5·85813	5·85261	5·85244	5·84784	5·84925	5·84520	5·84412	5·84405
40°	*E*	0·72869	0·70966	0·69854	0·69045	0·68468	0·67951	0·67526	0·67168
	y	5·86974	5·86461	5·86179	5·85652	5·86058	5·85657	5·85513	5·85551
45°	*E*	0·73401	0·71466	0·70326	0·69492	0·68922	0·68405	0·67975	0·67608
	y	5·88340	5·87802	5·87357	5·86639	5·87302	5·87032	5·86916	5·86902
50°	*E*	0·73928	0·71950	0·70805	0·69982	0·69366	0·68852	0·68413	0·68037
	y	5·89664	5·88929	5·88685	5·88343	5·88429	5·88332	5·88181	5·88116

Table 2 (*cont.*)

Set 3

$10^2 m_1$		1·18513	2·76632	5·92856	7·50974	9·09089	10·67212	12·25329
$10^2 m_2$		0·31072	0·72528	1·55435	1·96891	2·38346	2·79802	3·21258
$10^2 m_3$		0·58868	1·37410	2·94486	3·73027	4·51567	5·30110	6·08651
$10^6 m_{H^+}$	(35°)	5	5·5	6	7	7	7	8
$10^2 I$		0·89988	2·09991	4·49983	5·69984	6·89983	8·09985	9·29984
5°	*E*	0·65420	0·63337	0·61426	0·60824	0·60334	0·59914	0·59551
	y	5·83239	5·82355	5·80855	5·80216	5·79638	5·78994	5·78418
10°	*E*	0·65889	0·63766	0·61825	0·61214	0·60715	0·60287	0·59919
	y	5·82875	5·81947	5·80524	5·79917	5·79336	5·78684	5·78135
15°	*E*	0·66359	0·64196	0·62225	0·61604	0·61096	0·60663	0·60287
	y	5·82774	5·81801	5·80452	5·79859	5·79275	5·78668	5·78092
20°	*E*	0·66831	0·64628	0·62628	0·61997	0·61478	0·61039	0·60657
	y	5·83000	5·81985	5·80726	5·80147	5·79524	5·78944	5·78377
25°	*E*	0·67301	0·65061	0·63030	0·62388	0·61859	0·61413	0·61025
	y	5·83452	5·82447	5·81240	5·80657	5·80015	5·79443	5·78885
30°	*E*	0·67770	0·65492	0·63430	0·62780	0·62237	0·61789	0·61389
	y	5·84142	5·83129	5·81974	5·81436	5·80709	5·80228	5·79578
35°	*E*	0·68234	0·65919	0·63825	0·63169	0·62612	0·62159	0·61749
	y	5·85073	5·84070	5·82947	5·82486	5·81677	5·81235	5·80530
40°	*E*	0·68704	0·66344	0·64217	0·63552	0·62986	0·62536	0·62099
	y	5·86291	5·85169	5·84062	5·83628	5·82819	5·82543	5·81511
45°	*E*	0·69168	0·66765	0·64600	0·63930	0·63347	0·62890	0·62449
	y	5·87634	5·86427	5·85256	5·84911	5·83976	5·83703	5·82718
50°	*E*	0·69628	0·67185	0·64960	0·64298	0·63709	0·63259	0·62806
	y	5·88950	5·87708	5·86132	5·86075	5·85190	5·85138	5·84074

Table 3. *2-Aminoethanol 1-phosphoric acid, second dissociation. Observed and calculated values of* pK_{2a} *at rounded temperatures, together with '*β*' the slopes of the extrapolations,* $m_{R^{+-}}/m_{R^{+\pm}}$ *the buffer ratios, the constants of the equation* $pK_{2a} = A/T - D + CT$*, the minimum* pK_{2a} *and the temperature at which it occurs*

The calculated values of pK_{2a} are from the equation relating pK_{2a} to temperature, the standard deviation, σ, was calculated according to Please (1954). Values of pK_{2a} (obs.) are given to four decimal places, as these values were used to calculated A, C, and D and pK_2 (calc.).

	Set 1		Set 2		Set 3		
Temp. (°)	pK_{2a} (obs.)	$-'\beta'$	pK_{2a} (obs.)	$-'\beta'$	pK_{2a} (obs.)	$-'\beta'$	pK_{2a} (calc.)
5	5·8338	0·296	5·8401	0·166	5·8358	0·569	5·836
10	5·8293	0·281	5·8360	0·163	5·8319	0·557	5·832
15	5·8282	0·278	5·8348	0·163	5·8305	0·545	5·832
20	5·8297	0·264	5·8368	0·159	5·8326	0·536	5·834
25	5·8350	0·272	5·8415	0·160	5·8372	0·530	5·838
30	5·8420	0·273	5·8480	0·161	5·8441	0·525	5·845
35	5·8510	0·259	5·8578	0·160	5·8536	0·519	5·854
37	—	—	—	—	—	—	5·858
40	5·8634	0·277	5·8686	0·157	5·8653	0·525	5·865
45	5·8768	0·285	5·8808	0·147	5·8785	0·542	5·878
50	5·8898	0·289	5·8942	0·154	5·8901	0·529	5·893
$\frac{m_{R^{+-}}}{m_{R^{+\pm}}}$ (25°)	0·98		0·57		2·81		$\sigma = \pm 6 \times 10^4$

$A = 1228{\cdot}3378$; $C = 0{\cdot}01493$; $D = 2{\cdot}7328$; $pK_{min.} = 5{\cdot}832$; $t_{min.} = 13{\cdot}7°$.

Table 4. *2-Aminoethanol 1-phosphoric acid; second dissociation. Thermodynamic quantities*

Temp. (°)	$\Delta G°$ Joule	$\Delta G°$ Cal.	$\Delta H°$ Joule	$\Delta H°$ Cal.	$-\Delta S°$ Joule deg.$^{-1}$	$-\Delta S°$ Cal. deg.$^{-1}$	$-\Delta C_p°$ Joule deg.$^{-1}$	$-\Delta C_p°$ Cal. deg.$^{-1}$
5	31 077	7428	1402	335	106·6	25·5	159·0	38·0
10	31 617	7557	600	143	109·5	26·2	161·8	38·7
15	32 172	7689	−217	−52	112·4	26·9	164·7	39·7
20	32 741	7825	−1047	−250	115·2	27·5	167·6	40·1
25	33 325	7965	−1892	−452	118·1	28·2	170·4	40·7
30	33 923	8108	−2752	−658	120·9	28·9	173·3	41·4
35	34 535	8254	−3625	−866	123·8	29·6	176·1	42·1
37	34 783	8313	−3979	−951	124·9	29·9	177·3	42·4
40	35 161	8404	−4513	−1079	126·7	30·3	179·1	42·8
45	35 801	8557	−5415	−1294	129·5	31·0	181·9	43·5
50	36 456	8713	−6332	−1513	132·4	31·6	184·7	44·1
				Errors				
				σ_5, σ_{25}, σ_{50} = standard errors at 5, 25 and 50° respectively.				
σ_5	3·7	0·9	112	27	0·39	0·09	4·6	1·1
σ_{25}	2·2	0·5	34	8	0·11	0·03	5·0	1·2
σ_{50}	3·7	0·9	123	29	0·39	0·09	5·4	1·3

Table 5. *2-Aminoethanol 1-phosphoric acid.* pK_{1a}, pK_{2a} *and* pK_{3a} *at 20° on the molar scale*

These values were obtained from the pH titration curves (glass electrode) shown in Figs. 1 and 2. The number of points used in the calculations and the standard errors are also given.

$$pK_{1a} = pH + \log C_{R}/C_{R^{+-}} - AI^{\frac{1}{2}}/(1+I^{\frac{1}{2}});$$
$$pK_{2a} = pH + \log C_{R^{+-}}/C_{R^{+2}-} + AI^{\frac{1}{2}}/(1+I^{\frac{1}{2}});$$
$$pK^{*}_{2a} = pK_{2a} - \beta_2 I;\ \beta_2 \text{ being found from Fig. 3;}$$
$$pK_{3a} = pH + \log C_{R^{+2}-}/C_{R^{2-}} + 3AI^{\frac{1}{2}}/(1+I^{\frac{1}{2}});$$

$pK^{\dagger}_{2a}$ was found from cells without liquid junction corrected to the molar scale.

$\sigma\lambda$	pK	No. of points used	σ
pK_{1a}	<1	—	—
pK_{2a}	5·783	14	±0·021
pK^{*}_{2a}	5·831	14	±0·016
$pK^{\dagger}_{2a}$	5·835	—	±0·0006
pK_{3a}	10·89	13	±0·014

obtained at very different buffer ratios would not show such good agreement. As the differences between the three sets of results are not statistically significant, it may be assumed that they are mostly due to variations in the ester and errors in the experimental technique. It is, however, of interest to note that the slopes 'β' of the extrapolations vary markedly with the buffer ratio, indicating that there is some residual systematic effect on the activity coefficients of one or other of the ions which is not accounted for by the approximation used. Such marked variation of 'β' with the buffer ratio was not seen with the other phosphate esters which have been studied. The usefulness of the Guntelberg approximation for the activity coefficients, together with a linear term in I, in correcting results from glass electrode measurements of the pH titration curve to give the thermodynamic pK is well illustrated by the results in Table 5.

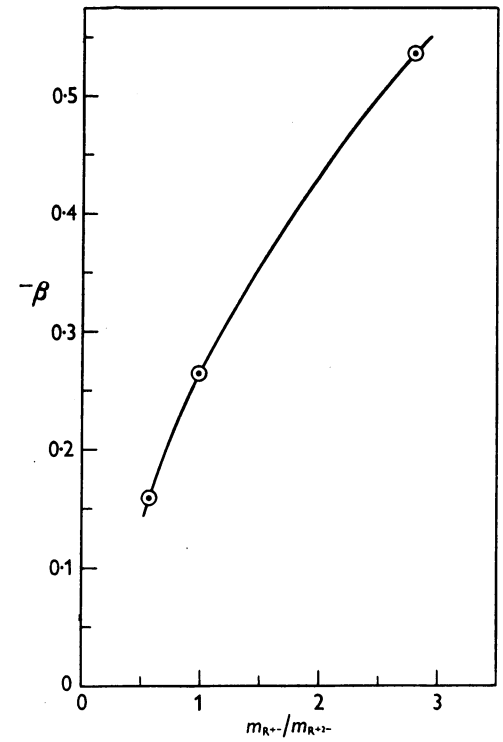


Fig. 3. Variation of the slopes 'β' of the extrapolations of the results from cells without liquid junction with the buffer ratio $m_{R^{+-}}/m_{R^{+2}-}$. The curve is from data given in Table 3.

The errors in the determination of both pK_{2a} and the thermodynamic quantities are similar to those found in the determinations of pK_{2a} of glycerol 2-phosphoric acid and glucose 1-phosphoric acid. This is surprising, since, in the present experiments,

Table 6. *The acid dissociation constants of 2-aminoethanol 1-phosphoric acid and a number of related compounds*

Substance	pK_{1a} (20°)	M	A	pK_{2a} (25°)	M	A	$pK_{NH_3^+}$ (20°)	M	A
2-Aminoethanol 1-phosphoric acid	<1	1	1	5·838	2	1	10·89	1	1
Glucose 1-phosphoric acid	1·23 (30°)	1	3	6·504	2	4	—	—	—
Glycerol 2-phosphoric acid	1·301	2	2	6·650	2	2	—	—	—
H_3PO_4	2·127	2	5	7·198	2	6	—	—	—
2-Aminoethanol	—	—	—	—	—	—	9·47 (25°)	3	8
Taurine	—	—	—	—	—	—	9·186	2	7

M = method

1 Glass electrode with liquid junction
2 Cells without liquid junction
3 H_2 electrode with liquid junction

A = author

1 This paper
2 Ashby, Crook & Datta (1954*b*)
3 Cori, Colowick & Cori (1937)
4 Ashby, Clarke, Crook & Datta (1955)
5 Bates (1951)
6 Bates & Acree (1943)
7 King (1953)
8 Simms (1928)

the solutions were made up with NaOH with the attendant errors in standardization, freedom from carbon dioxide contamination, etc.

From the results given in Table 6 it is seen that both the first and second acid dissociations of 2-aminoethanol 1-phosphoric acid are markedly stronger than those of the sugar phosphoric acids, which are themselves stronger than those of orthophosphoric acid. This increased strength of the two phosphate hydrogen dissociations over those of the sugar phosphates is presumably due to the powerful inductive effect of the positive charge on the nitrogen atom (cf. Kumler & Eiler, 1943). It is to be noted that the $pK_{NH_3^+}$ of 2-aminoethanol 1-phosphoric acid is considerably higher than that of taurine, again presumably due to the inductive effect of the two negative charges on the phosphate oxygens in place of the single negative charge on the sulphonic oxygens. This increased charge would be expected to impede the dissociation of the amino hydrogen.

The thermodynamic quantities associated with the second acid dissociation of 2-aminoethanol 1-phosphoric acid are similar to those for other ester phosphates, as is the temperature at which the maximum dissociation occurs.

SUMMARY

1. The second thermodynamic dissociation constant of 2-aminoethanol 1-phosphoric acid on the molal scale has been measured at 5° intervals from 5 to 50° using hydrogen and silver–silver chloride electrodes in cells without liquid junction.

2. From these measurements have been calculated the standard free energy, heat content, entropy and heat capacity for the ionization over the whole temperature range.

3. The first, second and third dissociation constants of 2-aminoethanol 1-phosphoric acid on the molar scale have been estimated at 20° from glass-electrode measurements with liquid junction.

4. It is demonstrated how the activity coefficients may be allowed for in the glass-electrode measurements.

5. The marked differences between the acid dissociation constants of 2-aminoethanol 1-phosphoric acid and those of similar acids are discussed.

The authors thank the Central Research Fund of the University of London for a grant to purchase the potentiometric equipment used in this work; also Mr P. A. T. Swoboda for assistance in the phosphate determinations and Mr V. L. Bengeri for much technical assistance. One of the authors (H.B.C.) thanks the Department of Scientific and Industrial Research, another of the authors (B.R.R.) thanks the Medical Research Council for personal grants.

REFERENCES

Ashby, J. H., Clarke, H. B., Crook, E. M. & Datta, S. P. (1955). *Biochem. J.* **59**, 203.
Ashby, J. H., Crook, E. M. & Datta, S. P. (1954*a*). *Biochem. J.* **56**, 190.
Ashby, J. H., Crook, E. M. & Datta, S. P. (1954*b*). *Biochem. J.* **56**, 198.
Bates, R. G. (1951). *J. Res. nat. Bur. Stand.* **47**, 127.
Bates, R. G. & Acree, S. F. (1943). *J. Res. nat. Bur. Stand.* **30**, 129.
Campbell, P. N. & Work, T. S. (1952). *Biochem. J.* **50**, 449.
Clarke. H. B., Cusworth, D. C. & Datta, S. P. (1954). *Biochem. J.* **58**, 146.
Cori, C. F., Colowick, S. P. & Cori, G. T. (1937). *J. biol. Chem.* **121**, 465.
DuMond, J. W. M. & Cohen, E. R. (1948). *Rev. mod. Phys.* **20**, 82.

DuMond, J. W. M. & Cohen, E. R. (1953). *Rev. mod. Phys.* **25**, 691.
Dunn, M. S. & Loshakoff, A. (1936). *J. biol. Chem.* **113**, 359.
Harned, H. S. & Ehlers, R. W. (1933). *J. Amer. chem. Soc.* **55**, 2179.
Harned, H. S. & Robinson, R. A. (1940). *Trans. Faraday Soc.* **36**, 973.
King, E. J. (1953). *J. Amer. chem. Soc.* **75**, 2204.
Kumler, W. D. & Eiler, J. J. (1943). *J. Amer. chem. Soc.* **65**, 2355.
Outhouse, E. L. (1937). *Biochem. J.* **31**, 1459.
Please, N. W. (1954). *Biochem. J.* **56**, 196.
Rossini, F. D., Gucker, F. T., Johnston, H. L., Pauling, L. & Vinal, G. W. (1952). *J. Amer. chem. Soc.* **74**, 2699.
Simms, H. S. (1928). *J. phys. Chem.* **32**, 1121.
Walker, D. M. (1952). *Biochem. J.* **52**, 679.

Toxicity of Aromatic Acids to the larvae of the Mosquito *Aedes aegypti* L. and the Counteracting Influence of Amino Acids

By J. E. CASIDA
Department of Entomology, University of Wisconsin, Madison, Wisconsin

(*Received* 28 *June* 1954)

Knowledge of the detoxication mechanisms of insects is becoming of ever-increasing importance. The metabolism of insecticides may so alter their biological activity as to destroy their toxicity (Sternburg & Kearns, 1950), produce new poisons in the plant from applied chemicals (Casida, Chapman, Stahmann & Allen, 1954; Metcalf, 1954) or even form the material which is toxic to insects from a non-toxic precursor (Metcalf & March, 1953; Casida & Stahmann, 1953). Locusts have been reported to contain the enzymes for glucuronide and ethereal sulphate hydrolysis (Robinson, Smith & Williams, 1953); they also form hippuric acid from benzoic acid (Friedler & Smith, 1953), glucosides from phenols (Myers & Smith, 1953*a*, 1954) and can acetylate aromatic amines (Myers & Smith, 1953*b*). In addition, insects can dehydrochlorinate certain chlorinated hydrocarbons enzymically (Sternburg, Vinson & Kearns, 1953), and oxidize thiophosphates (Metcalf & March, 1953) and dimethylphosphoramides (Casida, Allen & Stahmann, 1953). However, attempts to demonstrate methylation in *Bombyx mori* and *Lucilia caesar* by the isolation of *N'*-methylnicotinamide following the feeding of nicotinamide have proved unsuccessful (Kato, 1953).

Aromatic and amino acids play an important role in the life of insects. Aromatic acids may serve as egg-hatching factors for mosquitoes (Abdel-Malek, 1948; Gjullin, Yates & Stage, 1939), and as insecticides or repellents (Bushland, 1940; Fennah, 1950; Swingle, Phillips & Gahan, 1944; and others). Although the toxicity of a few aromatic acids has been studied with mosquito larvae (Bodine, 1923; Bushland & King, 1943), no detailed studies on the structural relationship for aromatic acid toxicity have been reported. In regard to amino acids, isoleucine is required for oviposition by adult *Aedes aegypti* (Greenburg, 1951) and the larvae require ten amino acids for growth (Goldberg & DeMeillon, 1948). Paper chromatograms have identified twenty amino acids from adult Culicid mosquitoes (Clark & Ball, 1952) and eighteen from all the developmental stages of *Aedes aegypti* L. (Micks & Ellis, 1951, 1952).

The recent identification of a conjugation product such as hippuric acid (Friedler & Smith, 1953) in locusts does not necessarily indicate that the toxicity of benzoic acid to the insect was actually reduced by this conjugation. The ease of studying a detoxication problem with mosquito larvae bioassay prompted the use of this technique. The investigation reported here concerns the toxicity of various aromatic acids to mosquito larvae with particular reference to the counteracting influence of amino acids.

MATERIALS AND METHODS

Mosquito larvae. A small colony of *Aedes aegypti* L. was maintained (Casanges, McGovern & Chiles, 1949) as a source of eggs, which were collected on moist filter paper and dried so that they could be hatched when desired for use. The first-instar larvae generally used for assay were not fed before testing; later instars were fed on finely ground dog biscuits (Friskies Dog Food Meal) until the actual assay time, when only the experimental nutrients were provided. Larval instars were differentiated on the basis of head capsule width. The adult mosquitoes were fed 5% sucrose solutions except for a single blood meal immediately after emergence.

Compounds. The amino acids and aromatic acids tested were of the highest purity obtainable commercially. The samples of D- and L-glutamic acids were optically pure. The identities of the substituted benzoic acids were ascertained by melting points and neutralization equivalents. All amino acids were tested at 0·1 M concentration unless specifically stated otherwise. In the study of hydrogen-ion concentration, a buffer which was 0·02 M with respect to each of