287. α-Amino-β-keto-acids. Part II.[1] Rates of Decarboxylation of the Free Acids and the Behaviour of Derivatives on Titration.

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Rates of decarboxylation of α-amino-β-oxo-butyric and -adipic acid have been measured in ethanol and in water at various pH's. Both acids have maximum half-lives of about 20 min. in ethanol and about 8 min. in water at pH 0. The half-lives decrease with increase in pH, and at pH 7·0 are much less than 1 min., being too short to be measured by either the volumetric or the spectrometric techniques employed.

The pK' values of the amino- and acetamido-keto-esters, and their ultraviolet absorption spectra, show that both types of compound form an enol ion very readily. This behaviour is discussed in relation to factors influencing the decarboxylation of the free acids.

In the preceding paper,[1] experiments were described in which benzyl ester salts of two α-amino-β-keto-acids (amino-oxo-butyric and -adipic acid) were treated in several ways in order to remove the ester group. In all experiments rapid decarboxylation occurred and only the α-amino-ketones were isolated. From the volume changes during catalytic debenzylation it was apparent that absorption of hydrogen could be made to occur faster than decarboxylation. It is shown in the present paper that both absorption of hydrogen and evolution of carbon dioxide follow first-order kinetics, and that a transient accumulation of the free acid can be demonstrated spectrophotometrically. The results show that in aqueous solution at room temperature the two amino-keto-acids have similar half-lives, of the order of a few minutes in acid solution. Since the temperature and, in certain cases, the pH during reaction were not kept constant, the values obtained are considered to be only approximate. The emphasis of investigation was on the possibility of isolating the free amino-keto-acids for use in biological systems, rather than on obtaining precise velocity constants.

Experimental

Materials.—Preparation of the compounds is described in the preceding paper.[1]

Volumetric Procedure.—Hydrogenations in which volume changes were measured were conducted at room temperature (22—24°) and atmospheric pressure on quantities of 5—10

[1] Part I, Laver, Neuberger, and Scott, preceding paper.

mmoles. The reaction flask had a side-arm with a stop-cock. In addition to being used for introduction of the material to be hydrogenated, the side-arm was connected by a flexible tube to a series of wash-bottles containing aqueous barium hydroxide when it was desired to determine the carbon dioxide evolved; a stream of hydrogen could then be passed through the apparatus while the flask was being shaken.

The required weight of 10% palladized charcoal [2] was suspended in the solvent and equilibrated with hydrogen before addition of the compound to be reduced. However, since methanol or ethanol may inflame if added to the catalyst in the presence of air, these solvents were added to the catalyst after the apparatus had been filled with hydrogen. In all cases equilibration was deemed sufficient when the hydrogen uptake was less than 0·2 ml./min.; this rate or less was attained after 20 minutes' shaking.

Spectrophotometric Procedure.—The following procedure was used to follow the change of spectrum in ethanol or in water containing 10% v/v of ethanol. Palladized charcoal (0·15 g.) were equilibrated first with nitrogen and then with hydrogen. A solution in ethanol (10 ml.) of about 0·3 mmole of α-benzyl hydrogen α-amino-β-oxo-adipate hydrochloride monomethanol solvate, or of the toluene-*p*-sulphonate, was then added rapidly and the mixture was shaken under hydrogen for 1 min. The catalyst was then rapidly filtered off under pressure through a sintered-glass disc; 2 ml. of the filtrate were mixed with 18 ml. of water or ethanol, and sufficient was at once transferred to an absorption cell; the pH of the remainder was measured by means of a glass electrode. Measurements of the absorption at 257·5 mμ were made in a Carey recording spectrophotometer (10 cm. cell) or in a Unicam SP. 500 spectrophotometer (1 cm. cell). The initial optical densities were only about 70—80% of the calculated values extrapolated to zero time. By using solutions of the unchanged benzyl ester, this was found to be due to retention of up to a third of the absorbing material on the catalyst. The effect was significant only when the weight of catalyst used was as great as that of ester.

The light-absorption constants were obtained from measurements with the Unicam SP. 500 spectrophotometer.

Determination of Velocity Constants.—The rates of hydrogenolysis followed first-order kinetics, and were proportional to the amount of catalyst present (see Fig. 1 and Table 1). The subsequent decarboxylation of the free acid is a first-order process, so the overall kinetics are those of two first-order reactions whose rate equations are readily integrable.

If the quantities of benzyl ester, free acid, and decarboxylated product are b, c, and d respectively after t min., then:

$$b = B \exp(-k_1 t) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where B is the initial quantity of ester;

$$c = B \frac{k_1}{k_2 - k_1} [\exp(-k_1 t) - \exp(-k_2 t)] \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

and

$$d = B - (b + c) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

k_1 and k_2 being the respective rate constants for debenzylation and decarboxylation.

(a) *Volumetric determinations of* k_2. Most of the measurements of b, c, and d were made, not directly, but by following the changes in volume of the gas phase as hydrogen was absorbed and carbon dioxide evolved. If V is the volume of hydrogen equivalent to B, then:

$$(V - v_H + v_O)/V = (b + d)/B \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

where v_H and v_O are the volumes absorbed or evolved after t min., and

$$(V - v_O)/V = (b + c)/B \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

The sum, $(b + d)$, can therefore be evaluated from the observed changes in volume, while $(b + c)$ can only be evaluated volumetrically by determination of v_H. It follows from equations (1) and (3) that $\log(b + d)$ will initially be effectively linear in t with slope k_1 provided $k_2 < k_1$. If k_2 is finite then it is always theoretically possible to make $k_2 < k_1$ by increasing the amount of catalyst; so k_1 can generally be evaluated and hence v_H and, by difference, v_O. It also follows from equations (1) and (2) that, for $k_2 < k_1$, $\log(b + c)$ will eventually become linear in t, with

[2] Harington and Randall, *Biochem. J.*, 1931, **25**, 1917.

slope k_2, after an initial period with slope $< k_2$, during which b is being converted into c, but with little change in $(b + c)$.

The linear phase of log $(b + d)$ was conveniently extended as a result of the solubility of carbon dioxide in the non-gaseous phases, so that v_0 could not be obtained at low values of t. Because of this partition of carbon dioxide between the phases, the volume of the gas phase did not return completely to its original value when reaction was virtually complete. It was assumed that the non-gaseous phases were saturated with an approximately constant volume of carbon dioxide once the sum $(b + d)$ had started to increase (see Fig. 1). Accordingly, for evaluation of v_0 at sufficiently large values of t, the difference between the initial and the final volume was added to the difference between $(V - v_H + v_0)$ and $(V - v_H)$. The validity of this approximation with respect to the determination of k_2 was borne out by direct determination as described below. However, the absolute values of $(b + c)$ obtained volumetrically were clearly too low (see Fig. 1), and it was never possible to recover more than about 70% of the carbon dioxide presumably liberated. Also, if $k_1 < k_2$, then k_2 cannot be calculated from the slope of log $(b + c)$, which will then approximate to k_1.

(b) *Spectroscopic determination of* k_2. Dimethyl α-amino-β-oxoadipate hydrochloride absorbed about five times more strongly in the region 250—265 mμ than the decarboxylated product, δ-aminolævulic acid, which possesses only the absorption [3] expected from the carbonyl chromophore. Decarboxylation, following removal of the α-ester group, is therefore attended by a decrease in optical density of the solution provided no other reactions take place. For the benzyl ester hydrochloride, however, this decrease in absorption is only about 20%, and for the toluene-*p*-sulphonate about 14%, because absorption due to the benzyl chromophores is virtually unaltered by hydrogenolysis. Extinction coefficients of the relevant compounds are included in Table 2. Even for the benzyl ester toluene-*p*-sulphonate, the changes in absorption sufficed to permit direct evaluation of k_2: by analogy with equation (5),

$$(b + c)/B = (D_t - D_\infty)/(D_0 - D_\infty) \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

where D_0, D_t, and D_∞ represent respectively the optical densities at 257·5 mμ initially, after t min., and finally. Further, by employing a large excess of catalyst, b could be made negligible; under such conditions determination of δ-aminolævulic acid at the end of the experiment showed that all the ester groups must have been cleaved before the spectrophotometric measurements were started.

δ-*Aminolævulic Acid.*—(a) *Isolation after hydrogenation.* α-Benzyl hydrogen α-amino-β-oxoadipate toluene-*p*-sulphonate (3·277 g.) in absolute ethanol (100 ml.) was hydrogenated in the presence of palladized charcoal (0·25 g.). The volume changes are shown in Fig. 1. When reaction had ceased the catalyst was removed and ether was added. The crystals formed had m. p. 182—182·5°, mixed m. p. 181·5—182° with δ-aminolævulic acid toluene-*p*-sulphonate (m. p. 182·5—183°). The latter was prepared from the hydrochloride by metathesis on an ion-exchange column (Deacidite FF in the toluene-*p*-sulphonate form), as described for benzyl hydrogen amino-oxoadipate in the preceding paper.[1] On examination by paper chromatography in butan-1-ol–water–acetic acid (63 : 27 : 10; upper phase), the product isolated after hydrogenation showed a single spot (R_F 0·5) identical with that of δ-aminolævulic acid toluene-*p*-sulphonate.

(b) *Determination after hydrogenation.* α-Benzyl hydrogen α-amino-β-oxoadipate hydrochloride methanol solvate (100 mg.) in absolute ethanol (10 ml.) was hydrogenated with palladized charcoal (150 mg.) for 1·5 min. The mixture was then rapidly filtered and 0·2 ml. of filtrate, corresponding to 6·0 μmoles of benzyl ester, was diluted with 50 ml. of 0·1M-potassium phosphate of pH 7·4, and the absorption was read immediately in a 1 cm. cell at 260 and 273 mμ. The optical densities of the solution were 0·030 and 0·025 and did not alter with time; the optical density of the starting material at the same concentration and pH was initially 0·420 at 273 mμ. The amount of aminolævulic acid present in the buffered solution after hydrogenation was estimated by the picrate method of Shuster [4] as modified by Laver, Neuberger, and Udenfriend [5] and also by the method of Mauzerall and Granick,[6] giving 5·4 and 5·75 μmoles respectively.

[3] Scott, Ph.D. Thesis, London, 1954.
[4] Shuster, *Biochem. J.*, 1956, **64**, 101.
[5] Laver, Neuberger, and Udenfriend, *ibid.*, 1958, **70**, 4.
[6] Mauzerall and Granick, *J. Biol. Chem.*, 1956, **219**, 435.

Catalytic Hydrogenation of α-Benzyl Hydrogen α-Acetamido-β-oxoadipate.—The ester (1·54 g.) in absolute ethanol (50 ml.) was added to palladized charcoal (1 g.) in ethanol (25 ml.) under hydrogen. Uptake of hydrogen was 1 equiv. in 5 min. Slow evolution of carbon dioxide was detectable after 16 min. in a parallel experiment. After 15 min. the catalyst was removed and the filtrate evaporated to dryness under reduced pressure in an air-bath. The residue had m. p. 67—80° without evolution of gas, raised to 93—95·5° after recrystallization from ethanol–ether, mixed m. p. 91·5—98° with δ-acetamidolævulic acid [7] (m. p. 92—94°). One equivalent of sodium hydroxide was required to make an aqueous solution of the product alkaline to phenolphthalein.

In a second experiment one equivalent of anhydrous sodium acetate in ethanol was added after 5 min. in such a way as to cause no change in the total volume of the system. Evolution of carbon dioxide was then rapid, with $t_{\frac{1}{2}} \sim 15$ min.

Spectrophotometric Titration.—The acetamido-compounds listed in Table 2 were titrated by using sodium phosphate buffer solutions (0·1M with respect to P). For the amino-keto-ester salts a special procedure was necessary as the optical density of solutions of these compounds sufficiently dilute for spectroscopic study decreased linearly with time, but independently of pH and, therefore, of the initial density; the density at 273 mμ became negligible after 1 hr. The required pH was obtained either by mixing a solution of the ester salt with a 0·1M-buffer solution or else by dilution of a 0·10M-solution of the ester salt to which sufficient 0·10M-sodium hydroxide had been added; in the latter case the ionic strength was very low and the results were not used in calculating the pK' values reported; the pH was always checked potentiometrically after absorption measurements. It was found that the rate of fading was related to the nature and concentration of the buffer in the following way: diethylbarbiturate (veronal) > phosphate > acetate > no buffer; concentrated > dilute. The fading was only important at great dilution ($<10^{-5}$M) since the same absorption and rate of fading were found after dilution of a freshly made 0·05M-solution as with one which had remained at the selected pH for 1 hr. before dilution (cf. Fig. 4). For measurement of pK' values density readings were made every minute for 20 min. after dilution of a strong solution of ester salt with a buffer solution; extrapolation of the straight line, obtained by plotting on Cartesian co-ordinates, evaluated D_{273} at zero time. Similar values at zero time were obtained by using different buffer systems at the same pH.

The significance of the time-dependent spectroscopic changes is not clear. The changes were not reversed by acidification to pH 1 and re-neutralizing, and no absorption due to a pyrazine ($\lambda_{max.}$ 290; ε 10,000 [1]) was observed until after the diluted solution had been exposed to the air for several days.

Results and Discussion

Two typical sets of observations made by the volumetric procedure are shown in Fig. 1, together with the graphical method of measuring the half-lives corresponding to k_1 and k_2. Comparison of curves *B* and *D* shows that the amount of catalyst used did not affect the rate of decarboxylation. Some determinations by the spectrophotometric procedure are shown in Fig. 2; under these conditions k_1 is made very large, and changes in absorption are due only to decarboxylation. The results of a series of such measurements in ethanol and in water at several pH values are given in Table 1. It is evident that there is very little difference in stability between the two α-amino-β-oxo-acids studied, either in ethanol or in water.

In the preceding paper it was reported that, while hydrogenation of ethyl α-phenylhydrazono-β-oxobutyrate or its *N*-acetyl derivative, in organic solvents containing two or more equivalents of hydrogen chloride, gave the amino-keto-ester in good yield, similar treatment of the benzyl esters gave aminoacetone. It may be seen from Fig. 3, curve *A*, that in such cases no transient accumulation of the amino-keto-acid was demonstrable. As the free phenylhydrazono-acid is a relatively stable compound it seems probable that the aniline formed in the reaction catalyses [8] the decarboxylation of the amino-acid to such an extent that its rate of decomposition is greater than both the rates of uptake of

[7] Neuberger, Scott, and Shuster, *Biochem. J.*, 1956, **64**, 137.
[8] Widmark and Jeppsson, *Skand. Arch. Physiol.*, 1922, **42**, 43.

hydrogen even when the concentration of aniline, as opposed to anilinium ion, is low; this conclusion is supported by the values in Fig. 3, curve *C*, from which it is evident that from the corresponding oxime the amino-keto-acid can accumulate. Three mols. of hydrogen were absorbed. The faster rate (Fig. 3, curve *B*) represents debenzylation to form eventually the free phenylhydrazono-acid,[1] as was shown by examination of the ether-soluble material present when hydrogenation was stopped after uptake of about one mol.; 78% of the theoretical amount of the free acid having the correct m. p. was so isolated. The slower rate represents cleavage of the phenylhydrazono-group with uptake of two

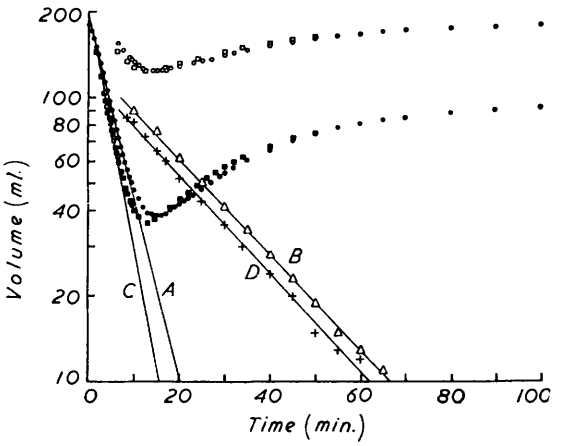


FIG. 1. *Determination of the rate of decarboxylation of amino-oxoadipic acid by the volumetric method.*

The results are derived from two experiments in ethanol, each with α-benzyl hydrogen α-amino-β-oxoadipate toluene-*p*-sulphonate equiv. to 180 ml. of hydrogen, with (1) 160 and (2) 250 mg. of palladized charcoal. In the notation of equation (4): $V = 180$; $b + d$ observed in expt. (1) ●, and in expt. (2) ■; $b + d$ corrected for retention of CO_2 in non-gaseous phases, in expt. (1) ○, and in expt. (2) □; hence $b + c$, in expt. (1) △, in expt. (2) +. k_1 and k_2 are evaluated, in terms of $t_{\frac{1}{2}}$, from the slopes of curves *A* and *B* [expt. (1)] and curves *C* and *D* [expt. (2)] (see Table 1).

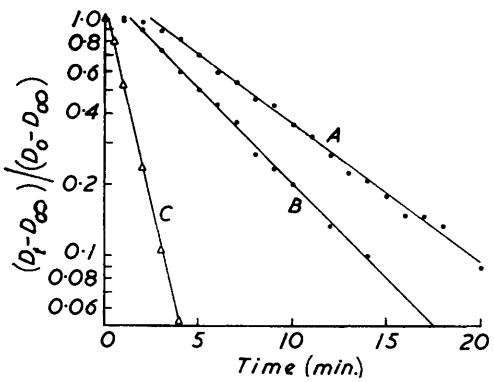


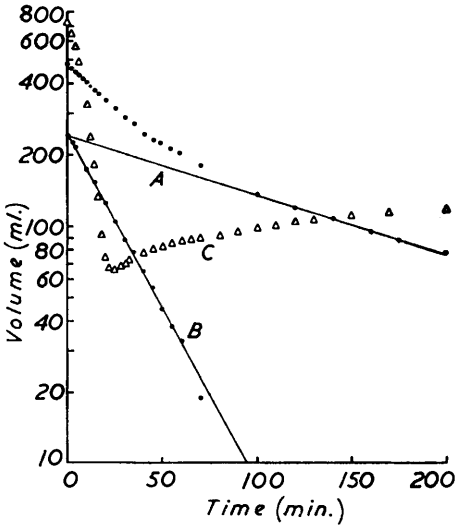
FIG. 2. *Determination of the rate of decarboxylation of amino-oxoadipic acid by the spectroscopic method.*

Aqueous solutions of the free acid were prepared as described. *A*, pH 3·30; *B*, pH 3·50; *C*, pH 5·0. See equation (6) and Table 1.

equivalents of hydrogen and output of one equivalent of carbon dioxide. 0·72 mol. of carbon dioxide was recovered as barium carbonate after the volume changes had ceased. Reductive cleavage of both the phenylhydrazono- and the hydroxyimino-group was found, from tests with the corresponding ethyl ester, to proceed at a single first-order rate. This suggests that adsorption of a molecule of the $>C{=}N^-$ compound on the catalyst is in effect followed by uptake of two equivalents of hydrogen before significant desorption takes place.

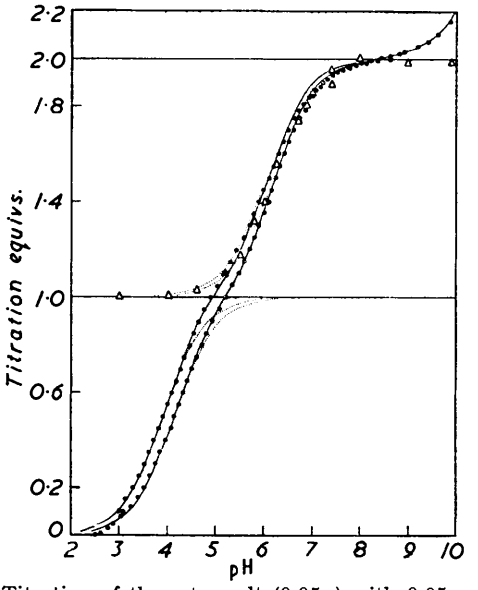
It appears from measurements of rates of decarboxylation at different pH values (Table 1) that the half-life of either amino-keto-acid decreases with increase in pH up to pH 5. At pH 7·0 and 7·4 the half-life was too short to be measured by either the volumetric or the spectrophotometric method. These values suggest that, while all forms of the acid (I; R = H) are unstable, one or more of the species formed on ionization is even less stable. Qualitatively, a similar decrease in stability on dissociation was observed for the corresponding *N*-acetyl free acids, the half-life in ethanol decreasing from several hours to about 15 min. when sodium acetate was added to the solution.

FIG. 3. *Hydrogenolysis of benzyl α-(N-acetyl phenylhydrazono)- and α-hydroxyimino-β-oxobutyrate.*



The volume change on hydrogenation is plotted analogously to that in Fig. 1. The ester indicated (10 mmoles) was hydrogenated in benzene containing dry HCl (30 mmoles) and palladized charcoal (1 g.). *N*-Acetylphenylhydrazone, observed values ○; curve *A* gives the slower rate of hydrogen uptake ($t_{\frac{1}{2}}$ 120 min.) and the difference is curve *B* (●), giving the faster rate ($t_{\frac{1}{2}}$ 21 min.). Oxime observed values △. Under the conditions of the experiments 10 mmoles of hydrogen measured 240 ml.

FIG. 4. *Potentiometric and spectrophotometric titration of α-benzyl hydrogen α-amino-β-oxoadipate toluene-p-sulphonate.*



Titration of the ester salt (0·05M) with 0·05N-NaOH, ●; the right-hand continuous and dotted lines give the theoretical values for pK' 4·15, 6·20 and, in part, 10·58. ○ represents the reverse titration with 0·05N-HCl after addition of 2·0 equivalents of 0·10N-NaOH; the left-hand continuous and dotted lines give the theoretical values for pK' 3·90 and 6·10. △, spectrophotometric titration (cf. Table 2); (D_{273} at pH 9) − (D_{273} at pH 3) = 0·8 (= 1 equiv.).

TABLE 1. *Approximate maximum half-lives (min.) of α-amino-β-oxo-acids.*

Solvent	pH	$t_{\frac{1}{2}}$ of free acid	$t_{\frac{1}{2}}$ of debenzn.	Solvent	pH	$t_{\frac{1}{2}}$ of free acid	$t_{\frac{1}{2}}$ of debenzn.
		α-Benzyl hydrogen α-amino-β-oxoadipate					
	Toluene-p-sulphonate				*Hydrochloride methanol solvate*		
EtOH	—	18·0 [a]	4·6 [c]	EtOH	—	15·0 [b]	⩽1
	—	17·5 [a]	3·1 [d]	H_2O	~0 [e]	>5<8 [b,f]	⩽1
	—	18·0 [b]	⩽1		3·30	5·0 [b]	⩽1
H_2O	2·5	6·7 [a]	5·0		3·50	3·7 [b]	⩽1
	3·28	5·2 [b]	⩽1		5·0 [g]	0·85 [b]	⩽1
					7·4	~0 [b,h]	⩽1
		Benzyl α-amino-β-oxobutyrate hydrochloride					
EtOH	—	22 [a]	2·5	H_2O	7N-HCl	8·1 [a]	1·85
					0·1N-HCl	7·7 [a]	1·5
					4·1	3·5 [a]	2·0
					4·9	1·85 [a]	1·35

[a] Volumetric method (Fig. 1). [b] Spectroscopic method (Fig. 2). [c,d] Using respectively 180 and 250 mg. of palladized charcoal. [e] N-HCl. The curve from which these values were obtained was not quite linear. [g] 0·05M-Buffer (acetic acid–sodium acetate). [h] Measurements were made at both 260 and 273 mμ.

Dissociation constants of the amino-keto-esters and their derivatives in water were determined either potentiometrically or by spectrophotometric titration. The results, expressed as pK' values of the relevant acidic groups, are summarized in Table 2, together with certain light-absorption constants.

For the acylamino-compounds listed in Table 2, pK' values of about 8 represent ionization of the enol, since no other group would be expected to be dissociating at this pH and also because the ionization is accompanied by a great increase in absorption at 276 mμ.

TABLE 2. pK' *values and absorption data for derivatives of α-amino-β-oxo-acids.*

Compound	pK' (22°) 1	2	3	Solvent	pH	$\lambda_{max.}$ (mμ)	ε
Et_2 α-amino-β-oxoadipate HCl				EtOH		257·5 [a]	98
	5·82 [c]	10·4 [c]		H_2O	3·9	260 [a]	99 [b]
	5·82 [d]				7·4	273	7600
α-CH_2Ph H α-amino-β-oxo-adipate HCl,MeOH	4·15	6·20 [c]	10·6 [c]	EtOH		257·5	394
		6·15 [d]		H_2O	3·3	257·5	394
					8·0	273	7910 [e]
α-CH_2Ph H α-amino-β-oxo-adipate toluene-*p*-sulphonate	4·15	6·20 [c]	10·6 [c]	EtOH		257·5	581
α-CH_2Ph H α-acetamido-β-oxo-adipate				EtOH		257	1280
				EtOH–NaOH [f]		276	23,100
	4·35	8·90 [c]		H_2O	6·3	260	256 [g]
		8·90 [d]			11·0	276	23,000
α-CH_2Ph H α-chloroacetamido-β-oxoadipate				EtOH		257	1212
				EtOH–NaOH [f]		276	21,700
CH_2Ph (or Et) α-amino-β-oxo-butyrate HCl	5·20 [c]	11·0 [c]			Not measured accurately		
Et α-chloroacetamido-β-oxo-butyrate				EtOH–NaOH [f]		274	17,200
	8·30 [c]			H_2O	6·0	252	550
	8·10 [d]				11·5	273	12,700
δ-Aminolævulic acid HCl [3]	4·05	8·90 [c]		H_2O	2·0	(257·5)	21 [h]

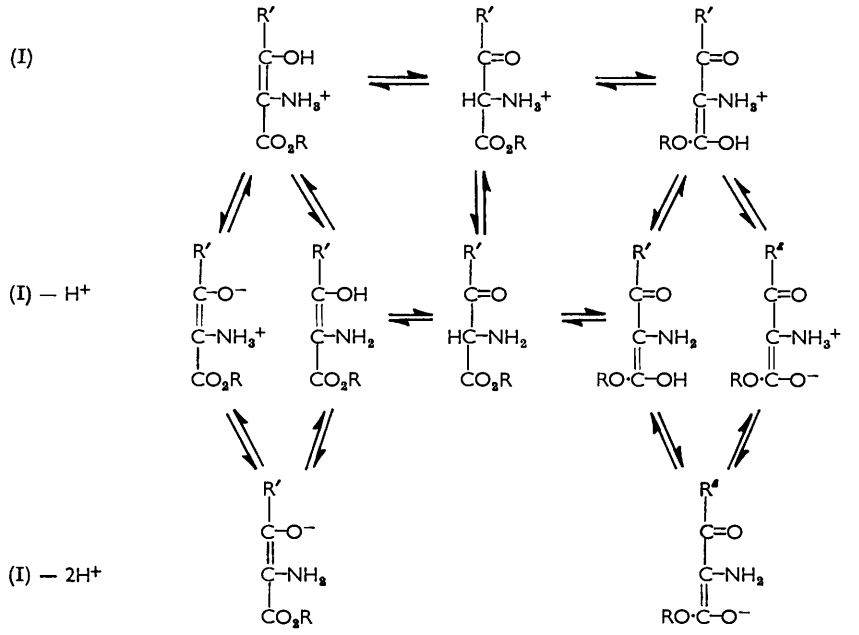
[a] Shoulder. [b] ε_{273} 89. [c] Potentiometric determination. [d] Spectroscopic determination. [e] Fig. 4. [f] 10^{-5}M-NaOH; absorbing compound $7·5 \times 10^{-6}$M. [g] ε_{276} 146. [h] This value is not $\lambda_{max.}$ but is given for comparison with $\varepsilon_{max.}$ of the α-amino-β-oxo-acids.

Similar changes of spectrum with pH were observed [9] in a series of the closely related penaldate esters ($\lambda_{max.}$ 265—270; ε *ca.* 15,000; apparent pK'_{enol} 6·3—6·6).[10] The true pK'_{enol} values must however be substantially lower than those observed, as the enol–keto-equilibrium in water favours the ketonic forms.

Direct titration of the toluene-*p*-sulphonate of the ester (I; R = CH_2Ph; R′ = CH_2·CO_2H) showed the presence of three ionizing groups. pK_1' has the value expected for the δ-CO_2H group, being similar to that of δ-aminolævulic acid; pK_3' presumably represents formation of structures equivalent to the free amino-enol ion, ·C(O^-):C(NH_2)·. Spectrophotometric titration showed the presence of only one operative group between pH 1 and 11, having the same value as pK_2'; measurements were made as described above, at 273 mμ, the value of $\lambda_{max.}$ for the absorbing species predominating at higher pH. Similarly the diethyl ester had two ionizing groups, only pK_1' being spectroscopically operative. In both cases potentiometric titration was almost completely reversible over a range of pH which included the spectroscopically operative pK' (Fig. 4) provided back-titration was carried out at once; solutions exposed to the air for several days at pH > (pK + 1) became cloudy and a crystalline precipitate of a sodium salt of the corresponding pyrazine [1] was formed slowly as the water evaporated.

Comparison with $\lambda_{max.}$ of the acylamino-compounds (Table 2) suggests the formation of an enol ion on titration of esters (I; R = alkyl), but $\varepsilon_{max.}$ is only one-half to one-third of the expected value. Without considerable further study it is not possible to define

[9] Merck and Co., Inc., 1944, Report 30; cited in " The Chemistry of Penicillin," p. 501, ed. Clarke, Johnson, and Robinson, Princeton Univ. Press, 1949.
[10] Eli Lilly and Co., 1944, Report 17; cited in *op. cit.*, p. 501.



Only *cis*-forms are shown.

pK_1' of the diester (I; R = Et, R′ = CH_2·CO_2Et) or pK_2' of the monoester (I; R = CH_2Ph, R′ = CH_2·CO_2H) with certainty in terms of the many equilibrium constants possibly involved. The equilibria must be complex because of the possibilities introduced, not only by keto–enol tautomerism, but also by the different acidities to be associated with the *cis*- and *trans*-forms of the amino-enol. Possible contributants to the equilibria are shown in the accompanying formulæ; only those forms are shown in which charges are localized on oxygen or nitrogen atoms. As the pK' in the region pH 10—11 is spectroscopically inoperative, it may be concluded that there is little change above pH 9 in the proportion of species having the αβ-unsaturated carbonyl structure. A net loss of a proton between pH 4 and 8 results therefore in complete enolization. The ratio of uncharged to zwitterionic enolic forms depends on a number of factors which cannot at present be assessed; in addition to those already mentioned, formation of five-membered rings involving hydrogen-bonds, such as those proposed for tropolone,[11] may have to be considered; in the present case the hydrogen-bond would lie between the amino-group and one of the two carbonyl groups. In view of the complexity of the system it is not advisable to draw conclusions from the marked decrease in value of ε_{273} from that for the corresponding *N*-acetyl compounds, although it may be noted that differences of a similar order have been observed in the case of the *K*-bands of *cis*- and *trans*-cinnamic acid.[12]

Table 1 shows that the half-life of amino-oxobutyric acid diminishes only slightly on rise of pH from <0 to about 1. The pK' of the carboxyl group would be expected to lie in the range 1—1·5 from consideration of the relevant pK' values of glycine and acetoacetic acid. Carbon dioxide must therefore be lost both from the mono-cation and from the zwitterion, R·CO·CH(NH_3^+)·CO_2^-, and at about the same rate. It may also be seen that the half-lives of both acids decrease sharply above pH 3 indicating that, above this pH, a species formed from the zwitterion by loss of a further one or two protons is very unstable. This species cannot be identified from the results so far obtained. Both the mono-cation and the zwitterion mentioned above possess a charged α-amino-group, and

[11] Cook and Loudon, *Quart. Rev.*, 1951, **5**, 99.
[12] Gillam and Stern, "Electronic Absorption Spectroscopy in Organic Chemistry," 1st edn., Arnold, London, 1954, p. 233.

the mechanism of decarboxylation may be similar to that proposed for the trichloroacetate ion,[13] rather than by the generally accepted mechanisms of decarboxylation of undissociated β-keto-acids.[14] As the very much less stable species cannot be identified, no mechanism is proposed to account for loss of carbon dioxide at less acid pH values.

The fact that decarboxylation is virtually instantaneous at neutral pH is of biochemical interest and indicates that amino-oxoadipic acid cannot be isolated from a biological system.

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[13] Verhoek, *J. Amer. Chem. Soc.*, 1934, **56**, 571; 1945, **67**, 1062; Hall and Verhoek, *ibid.*, 1947, **69**, 613; Cochran and Verhoek, *ibid.*, p. 2987; Auerbach, Verhoek, and Henne, *ibid.*, 1950, **72**, 299; Johnson and Moelwyn-Hughes, *Proc. Roy. Soc.*, 1940, *A*, **175**, 118.

[14] Pedersen, *J. Phys. Chem.*, 1934, **38**, 559; Westheimer and Jones, *J. Amer. Chem. Soc.*, 1938, **60**, 595; see also Brown, *Quart. Rev.*, 1951, **5**, 131.