The Dissociation Constants of Amino-acids in Deuterium Oxide

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Summary The isotope effect log K_H/K_D for the first dissociation constants of the amino-acids in the series $H_3N^+[CH_2]_xCO_2H$, $1 \leqslant x \leqslant 5$, and $[CH_3]_yN^+H_{3-y}\,CH_2CO_2H$, $0 \leqslant y \leqslant 3$, shows a good linear correlation with pK_H.

ALTHOUGH there are theoretical grounds[1,2] for believing that for a series of structurally related acids the dissociation constants in H_2O (K_H) and in D_2O (K_D) should be related by equation (1) in

$$pK_D - pK_H = \Delta pK = a + b pK_H \qquad (1)$$

which a and b are constants this is not, in general, supported by experimental results.[3] It has been shown[4] that although ΔpK and pK_H for some phenols and alcohols are linearly correlated, experimental results[4] for carboxylic acids provide little evidence for this relationship. We here report measurements which show that equation (1) holds for the ionisation of the carboxyl group in a series of structurally related acids.

We have determined the thermodynamic dissociation constants K $[=(L_3O^+)(Z^\pm)/(L_2O)(ZL^+)]$ (L = H or D) for the series of amino-acids for which $ZH^+ \equiv H_3N^+[CH_2]_xCO_2H$ (x = 1, 2, 3, and 5) and that for which $ZH^+ \equiv [CH_3]_yN^+H_{3-y}CH_2CO_2H$ (y = 1, 2, and 3), and also for DL-α-alanine. The values were obtained for solutions in ordinary water and a range of H_2O–D_2O mixtures, at 25°, by means of the cell,

Glass	$ZH^+Cl^-(m_1)$, $Z^\pm$ (m_2)	AgCl, Ag.
Electrode	(H_2O–D_2O)	

The "standard" e.m.f. required for the calculation of K was determined from measurements with hydrochloric acid in solvents of identical composition. Changes in the asymmetry potential of the glass electrode were taken into account. Measurements were made for a range of ionic strengths for each acid and solvent mixture and the thermodynamic dissociation constants were determined by extrapolation to zero ionic strength.[5] The method is capable of a precision of $\pm 0{\cdot}001$ and an accuracy of $\pm 0{\cdot}004$ log units. The values obtained for pK_H are in good agreement with those of previous workers, *e.g.* for glycine $pK_H = 2{\cdot}351$ *cf.* 2·350.[6]

The dissociation constants K_n obtained for 5 or 6 evenly spaced solvent deuterium atom fractions n (including one with $n > 0{\cdot}95$), along with the value for ordinary water, were fitted to equation (2)[7] by a least-squares procedure

$$K_n = K_H(1 - n + nl)^3/(1 - n + n\phi) \qquad (2)$$

which was used to determine K_H and ϕ (the fractionation factor for the carboxyl hydrogen). The value obtained for K_H agreed with the measured value within experimental error. The value of K_D given by equation (2) with $n = 1$, is insensitive to the value (0·69)[7] used for l (the fractionation factor for the lyonium ion). Although equation (2) is based on a number of assumptions[7] it was found to represent the experimental pK_n results with a standard deviation of 0·004 log units, and to provide a better basis for extrapolation than an empirical trinomial equation in n.

For glycine the value obtained for ΔpK (0·427) (molarity scale, as are all values here) is intermediate between those reported, at 25°, by earlier workers (0·54),[8] (0·39)[9] and for α-alanine our value (0·421) is below that (0·49)[8] found previously; isotope effects have not been reported for the other acids investigated.

The ΔpK values (Figure) obtained for all the acids except betaine ($y = 3$) are represented by equation (1) with $a = 0{\cdot}332$ and $b = 0{\cdot}040$. The low standard deviation (0·003 log units) and the high correlation coefficient (0·996) obtained for this regression line reflect the close structural similarities between the acids and in particular the fact that for the $H_3N^+[CH_2]_xCO_2H$ series the groups (NH_3^+, CO_2H, and CO_2^-) involved in hydrogen bonding with the solvent are the same for each acid. That ΔpK for betaine lies above the regression line by more than 0·02 units is almost certainly due to the different ways in which $-N(CH_3)_3^+$ and $-NH_3^+$ interact with the solvent.

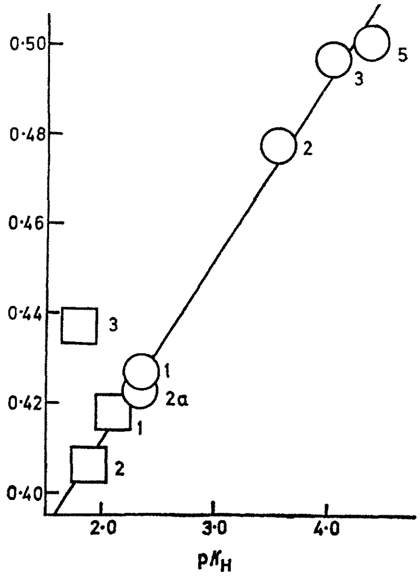

FIGURE. *Isotope effects* ΔpK *as a function of* pK_H. ○: *acids of type* $H_3N^+[CH_2]_xCO_2H$; □: *acids of type* $[CH_3]_yN^+H_{3-y}CH_2CO_2H$. *Numbers are values of* x *and* y. *Point* 2a *is for* DL-α-*alanine. The line represents equation* (1) *with* a = 0·332 *and* b = 0·040.

The slope ($b = 0{\cdot}040$) for the amino-acids is over twice that for phenols and alcohols ($b = 0{\cdot}017$);[4] this probably arises from the differing extents to which the phenoxide and carboxylate anions form hydrogen bonds with the solvent and is in agreement with a simple theoretical estimation, based on the method of Bunton and Shiner,[2] which gives $b = 0{\cdot}032$ for carboxylic acids and 0·020 for phenols.

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