# **484.** *Tracer Studies in Carboxylic Acids. Part IV.*[1] *Aminoacetic Acid*

By CHARMIAN O'CONNOR and D. R. LLEWELLYN

The rate of oxygen exchange of aminoacetic acid (glycine) varies with hydrogen-ion concentration. Four paths are postulated for this exchange. The four rate constants and their corresponding activation energies, frequency factors, and entropies of activation have been evaluated.

IN Part I [1b] of this investigation we dealt with the exchange of oxygen between acetic acid and pivalic acid with solvent water, and in Part II [1a] with the exchange of trifluoroacetic acid and trichloroacetic acid with solvent water. It was known that aminoacetic acid undergoes a slow oxygen exchange with water, catalysed by acid,[2] but no kinetic study had been undertaken previously. We now describe the exchange of the oxygen atoms of aminoacetic acid with solvent water over the hydrogen-ion concentration range 4—$10^{-12}$M and temperature range 25—123°.

*Mechanism of Oxygen Exchange.*—In an aqueous solution of aminoacetic acid the following equilibria are rapidly established:

$$^{+}NH_3{\cdot}CH_2{\cdot}CO_2H \overset{K_A}{\rightleftharpoons} {}^{+}NH_3{\cdot}CH_2{\cdot}CO_2^{-} + H^{+}$$

$$^{+}NH_3{\cdot}CH_2{\cdot}CO_2^{-} \rightleftharpoons NH_2{\cdot}CH_2{\cdot}CO_2H$$

$$^{+}NH_3{\cdot}CH_2{\cdot}CO_2^{-} \overset{K_B}{\rightleftharpoons} NH_2{\cdot}CH_2{\cdot}CO_2^{-} + H^{+}$$

and it can therefore be assumed that there are only four paths for the species in these equilibria to exchange oxygen atoms with water (no differentiation is made between $^{+}NH_3{\cdot}CH_2{\cdot}CO_2^{-}$ and $NH_2{\cdot}CH_2{\cdot}CO_2H$):

(1) $^{+}NH_3{\cdot}CH_2{\cdot}CO_2H + H^{+} + H_2O$

(2) $^{+}NH_3{\cdot}CH_2{\cdot}CO_2H + H_2O$ or $^{+}NH_3{\cdot}CH_2{\cdot}CO_2^{-} + H^{+} + H_2O$

(3) $^{+}NH_3{\cdot}CH_2{\cdot}CO_2H + OH^{-}$ or $^{+}NH_3{\cdot}CH_2{\cdot}CO_2^{-} + H_2O$

(4) $^{+}NH_3{\cdot}CH_2{\cdot}CO_2^{-} + OH^{-}$ or $NH_2{\cdot}CH_2{\cdot}CO_2^{-} + H_2O$

In each of reactions (2), (3), and (4) the two possibilities are mechanistically different but cannot be distinguished chemically at the present time.

This treatment neglects exchange due to attack on $NH_2{\cdot}CH_2{\cdot}CO_2^{-}$ by a hydroxyl ion because it was shown experimentally that even at pH >11, where $NH_2{\cdot}CH_2{\cdot}CO_2^{-}$ is the only species present, an increase in hydroxyl-ion concentration did not increase the rate. It also neglects any second-order terms in $^{+}NH_3{\cdot}CH_2{\cdot}CO_2H$ (*i.e.*, any general acid-catalysis) because the rate is found to be independent of the stoicheiometric concentration, $C$, of total glycine added.

If $\alpha_1$ is the fraction of the stoicheiometric concentration present as $^{+}NH_3{\cdot}CH_2{\cdot}CO_2H$, $\alpha_2$ as $^{+}NH_3{\cdot}CH_2{\cdot}CO_2^{-}$, and $\alpha_3$ as $NH_2{\cdot}CH_2{\cdot}CO_2^{-}$, then the rate of exchange is:

$$R = [H_2O]C(k_1\alpha_1[H^+] + k_2\alpha_1 + k_3\alpha_2 + k_4\alpha_3)$$

The rate constants $k_1$—$k_4$ refer to paths (1)—(4) above. Let $R/[H_2O]C = R^*$. Then

$$R^* = k_1\alpha_1[H^+] + k_2\alpha_1 + k_3\alpha_2 + k_4\alpha_3$$

---

[1] (*a*) O'Connor and Llewellyn, *J.*, 1965, 2197; (*b*) Part II, Llewellyn and O'Connor, *J.*, 1964, 4400; (*c*) Part I, Llewellyn and O'Connor, *J.*, 1964, 545.

[2] (*a*) Mears and Sobotka, *J. Amer. Chem. Soc.*, 1939, **61**, 880; (*b*) Mears, *J. Chem. Phys.*, 1938, **6**, 295.

Allowance has been made for variation of the dissociation constants, $K_A$ and $K_B$, of glycine with temperature, by using the empirical relation:[3]

$$\log K = \log K_m - 5{\cdot}0 \times 10^{-5}(t - \theta)^2$$

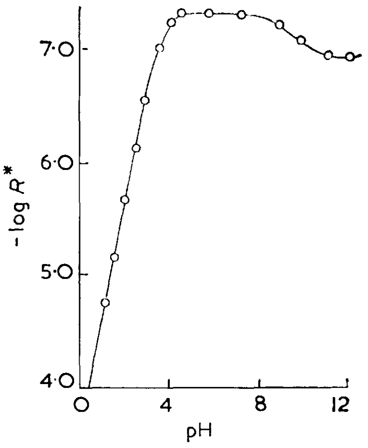
where $\theta$ is the temperature at which $K_A$ and $K_B$ are maxima, *i.e.*, equal to $K_m$. For $K_A$, $\theta = 53{\cdot}9°$ and $\log K_m = -2{\cdot}309$; for $K_B$, $\theta = 93{\cdot}0°$ and $\log K_m = -3{\cdot}988$. These values are calculated from the known[4] variations of $K_A$ and $K_B$ with temperature.

The values of $\alpha$ can be calculated from these dissociation constants and from the measured hydrogen-ion concentration, thus:

$$\alpha_1 = [H^+]^2/([H^+]^2 + [H^+]K_A + K_AK_B)$$
$$\alpha_2 = [H^+]K_A/([H^+]^2 + [H^+]K_A + K_AK_B)$$
$$\alpha_3 = K_AK_B/([H^+]^2 + [H^+]K_A + K_AK_B)$$

The McKay plots[5] for exchange were linear over the range studied, up to 92% of complete exchange.

The experimental values of $R^*$ are shown in Table 1. The fact that the runs at $T = 101{\cdot}0°$, pH 2·12, and at $T = 123{\cdot}0°$, pH 2·55, where the concentration of amino-acetic acid was 0·075M, fit the series for which $C = 0{\cdot}3$M, shows that the rate of $^{18}O$ exchange depends solely on the difference in isotopic abundance of the two species, aminoacetic acid and water, and is independent of their concentration. Thus the possibility of catalysis of exchange by $^+NH_3{\cdot}CH_2{\cdot}CO_2H$ can be discounted.



Dependence of rate of aminoacetic acid–$H_2{}^{18}O$ exchange on pH; $I = 1$; 123·0°

A typical pH profile of the rate of exchange (*i.e.*, —log $R^*$ plotted against pH) is shown in the Figure.

When $[H^+] > 1$M, $^+NH_3{\cdot}CH_2{\cdot}CO_2H$ is the only species present, *i.e.*, $\alpha_1 = 1$ and $R^* = k_1[H^+] + k_2$.

At pH 1—4·5 the species present are $^+NH_3{\cdot}CH_2{\cdot}CO_2H$ and $^+NH_3{\cdot}CH_2{\cdot}CO_2^-$, and $R^* = k_1\alpha_1[H^+] + k_2\alpha_1 + k_3\alpha_2$.

The only species present at pH 4·5—7·5 is $^+NH_3{\cdot}CH_2{\cdot}CO_2^-$, *i.e.*, $\alpha_2 = 1$ and $R^* = k_3$.

At pH > 11, the only species present is $NH_2{\cdot}CH_2{\cdot}CO_2^-$, *i.e.*, $\alpha_3 = 1$ and $R^* = k_4$. The values of $k_1$, $k_2$, $k_3$, and $k_4$ are shown in Table 2. In order to show the adequacy of the analysis, the values of $R^*$ calculated from the tabulated values of $k_1$—$k_4$ ($R^*_{calc}$), are given in Table 1. The rate constant $k_1$, calculated from results of experiments with no

---

[3] Harned and Embree, *J. Amer. Chem. Soc.*, 1934, **56**, 1050.
[4] Owen, *J. Amer. Chem. Soc.*, 1934, **56**, 24.
[5] McKay, *Nature*, 1938, **142**, 997.

added salt, is larger than the corresponding $k_1$ at unit ionic strength. This result, which indicates a negative salt effect, is surprising, because the primary salt effect on the dissociation of aminoacetic acid should give a positive result. However, it may be that the effect of the salt on the glass electrode is greater than the effect of the salt on the rate.

The Arrhenius plot of log $k_1$ against $1/T$ was linear. Hence the frequency factor, $A$, the activation energy, $E$, and the entropy of activation, $\Delta S^*$, could be evaluated. These parameters are also given in Table 3 for the other rate constants, but their significance is doubtful because of the small temperature range over which they are evaluated. No assessment has been made of the effect, if any, of temperature on pH, nor of added salt on the glass electrode; [H$^+$] was calculated directly from pH $= -\log_{10}$ [H$^+$], without consideration of activity coefficients. These assumptions and omissions are not entirely justified but the division of the exchange rate between the various paths does not depend upon them quantitatively to any great extent. The evaluation of $k_3$ and $k_4$ directly from regions of the pH profile where the rate of exchange is independent of pH does not involve [H$^+$], and so the results will not be affected by any slight displacement of the profiles.

TABLE 1

Exchange for 0·3M-aminoacetic acid–$H_2{}^{18}O$

No salt added

| Temp. | 76·0° | 76·0° | 76·0° | 76·0° | 100·0° | 100·0° | 100·0° | 100·0° | 100·0° | 100·0° | 100·0° |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 0·57 | 1·02 | 1·36 | 1·48 | 1·02 | 1·39 | 1·66 | 1·92 | 2·18 | 2·41 | 2·94 |
| $10^7R^*$ | 52·6 | 20·4 | 9·15 | 6·56 | 84·5 | 35·3 | 16·7 | 9·40 | 5·62 | 3·25 | 1·01 |
| $10^7R^*_{\text{calc.}}$ | 58·2 | 20·0 | 8·68 | 7·19 | 85·0 | 34·4 | 17·0 | 8·40 | | | |

At $I$ = 1.

| Temp. | 55·0° | 55·0° | 55·0° | 78·0° | 78·0° | 78·0° | 78·0° | 101·0° | 101·0° | 101·0° † | 101·0° |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1·34 | 1·85 | 2·23 | 1·24 | 1·85 | 2·23 | 2·52 | 1·24 | 1·85 | 2·12 | 2·23 |
| $10^8R^*$ | 12·8 | 3·23 | 1·42 | 76·5 | 19·9 | 9·24 | 4·78 | 350 | 85·3 | 43·9 | 43·1 |
| $10^8R^*_{\text{calc.}}$ | 13·9 | 2·81 | | 86·0 | 16·0 | | | 361 | 84·1 | 43·1 | 31·1 |

| Temp. | 101·0° | 101·0° | 101·0° | 101·0° | 101·0° | 101·0° | 101·0° | 101·0° | 101·0° | 101·0° | 101·0° |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 2·61 | 2·63 | 2·93 | 3·29 | 3·60 | 3·87 | 4·07 | 5·79 | 7·28 | 8·88 | 9·87 |
| $10^8R^*$ | 17·4 | 17·3 | 7·95 | 3·06 | 1·98 | 1·06 | 1·08 | 0·506 | 0·483 | 0·658 | 1·06 |
| $10^8R^*_{\text{calc.}}$ | 12·1 | 11·9 | 8·46 | 2·53 | 1·48 | 1·01 | 0·82 | 0·495 | 0·495 | 0·555 | 0·86 |

| Temp. | 101·0° | 101·0° | 123·0° | 123·0° | 123·0° | 123·0° † | 123·0° | 123·0° | 123·0° | 123·0° | 123·0° |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 11·13 | 12·09 | 1·06 | 1·51 | 2·06 | 2·55 | 2·61 | 2·93 | 3·60 | 4·09 | 4·51 |
| $10^8R^*$ | 1·38 | 1·34 | 1770 | 700 | 214 | 77·0 | 75·8 | 28·1 | 10·1 | 5·86 | 4·72 |
| $10^8R^*_{\text{calc.}}$ | 1·36 | 1·36 | 1870 | 699 | 201 | 69·0 | 60·6 | 32·1 | 14·8 | 11·7 | 4·90 |

| Temp. | 123·0° | 12·30° | 123·0° | 123·0° | 123·0° | 123·0° |
|---|---|---|---|---|---|---|
| pH | 5·79 | 7·28 | 8·88 | 9·87 | 11·13 | 12·09 |
| $10^8R^*$ | 4·91 | 5·02 | 6·22 | 9·31 | 11·7 | 12·5 |
| $10^8R^*_{\text{calc.}}$ | 4·90 | 4·90 | 5·31 | 8·10 | 12·1 | 12·1 |

At $I$ = 4.

| Temp. | 25·0° | 25·0° | 25·0° | 25·0° | 55·0° | 55·0° | 55·0° | 55·0° |
|---|---|---|---|---|---|---|---|---|
| [H$^+$] | 3·696 | 2·772 | 1·848 | 0·924 | 3·696 | 2·772 | 1·848 | 0·924 |
| $10^7R^*$ | 7·04 | 5·00 | 3·47 | 1·64 | 95·0 | 66·4 | 47·8 | 22·5 |
| $10^7R^*_{\text{calc.}}$ | 6·80 | 5·10 | 3·40 | 1·70 | 92·3 | 69·2 | 46·3 | 23·1 |

† Aminoacetic acid = 0·075M; $R^*$ in l. mole$^{-1}$ sec.$^{-1}$; $I$ denotes ionic strength.

The calculated values of $k_1$ at 25·0, 55·0, 76·0, 78·0, and 100·0° are based only on rate measurements in solutions where [H$^+$] was checked by acid–base titration. The values given for $k_1$ at 101·0 and 123·0° seem to be justified by the fact that they fit the Arrhenius plot extrapolated from the lower temperatures very well, and also a smooth curve can be drawn through the rates obtained at pH $\gtrsim 2$, where two different methods were used for determining [H$^+$].

The rate constant $k_2$ could, however, be affected by a displacement in the pH profile, but in comparison with $k_1$ it is of doubtful significance.

TABLE 2

Rate constants for $^{18}$O exchange of aminoacetic acid

| Temp. | $10^6k_1$ (l.$^2$ mole$^{-2}$ sec.$^{-1}$) | $10^7k_2$ (l. mole$^{-1}$ sec.$^{-1}$) | $10^9k_3$ (l. mole$^{-1}$ sec.$^{-1}$) | $10^8k_4$ (l. mole$^{-1}$ sec.$^{-1}$) | Conditions |
|---|---|---|---|---|---|
| 25·0° | 0·184 | | | | $I = 4$ |
| 55·0 | 2·50 | | | | ,, |
| 55·0 | 2·70 | | | | $I = 1$ |
| 78·0 | 16·1 | | | | ,, |
| 101·0 | 65·1 | 1·41 | 4·95 | 1·36 | ,, |
| 123·0 | 220 | 7·31 | 49·0 | 12·1 | ,, |
| 76·0 | 22·0 | | | | No salt added |
| 100·0 | 92·5 | | | | ,, |

TABLE 3

Arrhenius parameters

| Rate constant | Conditions | $E$ (kcal. mole$^{-1}$) | og $A$ | $\Delta S^*$ (cal. deg.$^{-1}$ mole$^{-1}$) |
|---|---|---|---|---|
| $k_1'$ | No salt added | 17 | 6 | −34 |
| $k_1''$ | $I = 1$ and $I = 4$ | 16 | 6 | −34 |
| $k_2'$ | $I = 1$ | 19 | 4 | −41 |
| $k_2''$ | ,, | 22 | 10 | −14 |
| $k_3'$ | ,, | 31 | 12 | −6 |
| $k_3''$ | ,, | 27 | 20 | +34 |
| $k_4'$ | ,, | 30 | 11 | −9 |
| $k_4''$ | ,, | 30 | 15 | +7 |

For convenience the rate constants (Table 2) were calculated so that they did not include $[H_2O]$, but the Arrhenius parameters (see Table 3) were calculated from the conventional rate constants, $k'$, where $k' = k[H_2O] = 55{\cdot}5k$.

The Arrhenius parameters for paths (2), (3), and (4) depend on whether the rate constant is calculated as $k'$ or $k''$,

where
$$R_2 = k_2'[^+NH_3CH_2CO_2H] \text{ or } k_2''[^+NH_3CH_2CO_2^-][H^+]$$
$$R_3 = k_3'[^+NH_3CH_2CO_2^-] \text{ or } k_3''\ [^+NH_3CH_2CO_2H][OH^-]$$
$$R_4 = k_4'[NH_2CH_2CO_2^-] \text{ or } k_4''[^+NH_3CH_2CO_2^-][OH^-]$$

The parameters for all rate constants are given in Table 3.

## EXPERIMENTAL

*Materials.*—The solvent was water containing ~1 atom-% abundance $^{18}$O. Aminoacetic acid (Hopkins and Wilkins) was recrystallised from water. Recrystallised lithium perchlorate was used to adjust the ionic strength. Acid solutions with $[H^+] > 0{\cdot}01$M were made up by mixing 60% AnalaR perchloric acid with water. The acid concentration was checked by acid–base titration. The pH of other solutions was adjusted by adding a few drops of 60% AnalaR perchloric acid or of a solution of AnalaR lithium hydroxide, and measured on a Doran pH meter.

*Kinetics of Oxygen Exchange.*—(*a*) *Solutions of* pH $>2$. Weighed samples of aminoacetic acid were dissolved by shaking them in solvent (sometimes containing perchlorate salt). After adjustment of the pH, aliquot parts (0·5 ml.) sealed in ampoules were placed in a constant-temperature bath.

(*b*) *Solutions of* $[H^+] > 0{\cdot}01$M. Weighed samples of aminoacetic acid were dissolved by shaking them in solvent containing perchloric acid and perchlorate salt, which was at the temperature of the thermostat-bath.

Samples were removed at intervals, cooled rapidly in liquid air, and brought to ~pH 7 (colourless to phenolphthalein) by the addition of dilute perchloric acid to base solutions and dilute lithium hydroxide to acidic solutions. A few drops of silver nitrate were added, and the resulting solution poured into 20 times its volume of acetone. Silver aminoacetate was precipitated, filtered off, washed with alcohol, dried *in vacuo*, and decomposed to carbon dioxide by gentle heating *in vacuo*. The carbon dioxide was analysed on an A.E.I.M.S. 3 mass spectrometer.

The initial abundance of $^{18}O$ in aminoacetic acid was measured on an unlabelled sample of silver salt, and excess abundances were calculated relative to this value. For the fast reactions the infinite-time value for complete exchange was measured after 8—10 half-lives. If $t_{1/2}$ was greater than 4 days, the infinite-time abundance was measured on a sample of carbon dioxide which had been in equilibrium with a portion of the exchange solution for 36 hr. at 100°.

The authors thank Dr. C. A. Bunton for many helpful comments and the Research Committee of the N.Z. University Grants Committee for a grant to purchase the mass spectrometer.

CHEMISTRY DEPARTMENT, UNIVERSITY OF AUCKLAND,
AUCKLAND, NEW ZEALAND.

[*Received, April* 6*th*, 1964.]