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A New Technique for Determination of the Immediate Product of Decarboxylation Reactions

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A new technique to determine whether decarboxylation reactions yield CO_2 or H_2CO_3 (HCO_3^-) as the immediate product is described. Air is passed at a high constant rate through a reaction vessel, where a carboxyl-^{14}C-labelled carboxylic acid is submitted to a decarboxytion reaction under well-defined conditions (pH, temperature), and the $^{14}CO_2$ removed is quantitatively collected. The total radioactivity of the collected $^{14}CO_2$ is determined as a function of time, and a reliable differentiation of the immediate products of the decarboxylation reaction can be made from the experimental data by means of a simple plotting method. The technique has partly been tested by model experiments, and partly been used to show that orsellinic acid decarboxylase from *Gliocladium roseum* functions as a carboxy-lyase.

Enzymes carrying out the decarboxylation of carboxylic acids can be classified as lyases or as hydrolases, depending on whether CO_2 or H_2CO_3 (HCO_3^-) is the immediate reaction product.[1] Owing to the rapid interconversion of these products it has been difficult with conventional methods to determine which is primarily formed, and decarboxylases are often classified as carboxy-lyases without proper experimental demonstration that CO_2 is the immediate end-product. A few techniques to determine the primary product of decarboxylation reactions have, however, been elaborated, which make use of the fact that the interconversion of H_2CO_3 and CO_2 (proceeding at a measurable rate under physiological conditions) is enzymatically catalyzed by carbonic anhydrase.[2] Roughton *et al.* thus used carbonic anhydrase as a tool in manometric studies of the urea-urease system to show that CO_2 is the immediate end-product.[3] In these experiments the relation of urease to urea was such that total urea-splitting occurred in a very short time, producing an initial rapid rise of CO_2 pressure and a gradual return to equilibrium. In the presence of carbonic anhydrase no such rise occurred. This method was further developed by Conway and O'Malley, who showed that the steady-state rate of CO_2 production in manometric assays of decarboxylases is altered in a mathematically predictable way by the addition of carbonic anhydrase, depending on whether the primary product is H_2CO_3 (HCO_3^-) or CO_2.[4] The above methods were found

to require pure and powerful decarboxylase preparations to make it feasible to differentiate the end-products, and the results obtained were considerably influenced by the experimental conditions.

A more useful and reliable method to distinguish between CO_2 and H_2CO_3 as primary decarboxylation products was elaborated by Palmer.[5] This method is dependent upon the removal of CO_2 by flowing air at different rates through reactants, and measuring the time required to collect a definite amount of liberated CO_2. Experimental conditions can be chosen in such a way that if H_2CO_3 (or HCO_3^-) is the primary product the removal of CO_2 is limited by the rate of the dehydration reaction. The rate of CO_2 liberation will thus at high air flows be relatively unaffected by flow rate, but strongly increased by the addition of carbonic anhydrase. If, on the other hand, CO_2 is the primary product increase of air flow rate will increase the rate of liberation of CO_2 (the rate-limiting factor being its production by the decarboxylation process) and addition of carbonic anhydrase will decrease the rate of CO_2 liberation.

None of the above methods is suitable for the study of decarboxylation reactions that are much slower than the dehydration of H_2CO_3, or which simultaneously yield both CO_2 and H_2CO_3 (HCO_3^-) as immediate products. Furthermore, they cannot be used in systems containing or producing CO_2 or HCO_3^- derived from other sources than the decarboxylation process under investigation. The work of Conway and O'Malley also showed that results obtained by methods based on the use of carbonic anhydrase may be dependent on the origin and purity of the carbonic anhydrase preparation.[4] In the present paper a new technique is described, which eliminates all of the above disadvantages. The technique, which is based on the principles elaborated by Palmer,[5] introduces the use of carboxyl-^{14}C-labelled carboxylic acids to determine the immediate products of enzymatic or non-enzymatic decarboxylation reactions. The radioactive CO_2 (ultimately derived from the labelled carboxylic acid) liberated by flowing air at a high constant rate through reactants is quantitatively collected and measured as a function of the reaction time. It will be shown that a simple method of plotting the experimental data permits a reliable differentiation of the immediate products to be made, without studying the effect of carbonic anhydrase on the process.

The technique has partly been tested by model experiments in which an enzyme-substrate system producing H_2CO_3 or HCO_3^- was simulated by the slow constant addition of $NaH^{14}CO_3$ to a buffer solution, and partly been used to show that orsellinic acid decarboxylase from *Gliocladium roseum* [6] yields CO_2 as the immediate decarboxylation product.

EXPERIMENTAL

Apparatus. The apparatus used was similar to that described by Palmer.[5] The reaction chamber consisted of a chromatographic column (20 × 2 cm) through which air previously passed through a barium hydroxide cannister was allowed to flow at a constant rate. The flow rate was measured by means of a calibrated flow meter. Air was dispersed into minute bubbles by a sintered-glass filter in the bottom of the column, flowed through the reactants (usually enzyme, radioactive substrate, and phosphate buffer), and was finally passed through a gas washing bottle containing Hyamine hydroxide in order to

quantitatively collect the radioactive CO_2 blown off from the reaction vessel. Solutions could be added rapidly through a syringe to the termostated reaction vessel during constant air flow.

A typical experiment began with an accurately measured flow of air (11.0 ml/sec) passing through the reaction vessel (+5°) containing orsellinic acid decarboxylase (about 0.1 enzymatic units) dissolved in 8 ml of 0.5 M phosphate buffer, pH 7.8. The syringe contained 5 mg of carboxyl labelled orsellinic acid (0.28 μC/mg) dissolved in 2 ml of the same buffer solution. At zero time the syringe was emptied into the reaction vessel. Small samples (1.0 ml) of Hyamine hydroxide were then withdrawn from the collecting vessel at definite intervals (usually 1 min), and the radioactivity of each sample was determined in a liquid scintillation counter after dilution with 15 ml of a 0.5 % solution of diphenyloxazol in toluene. The total radioactivity of the CO_2 removed by the air stream after different times was then obtained by elementary calculation; the collecting vessel contained 50 ml of Hyamine hydroxide at zero time.

Model experiments with bicarbonate. The hypothetical situation in which an enzyme-substrate system produces H_2CO_3 or HCO_3^- was simulated as described by Palmer.[5] A solution of $NaH^{14}CO_3$ was added through a syringe at a slow constant rate (0.08 μC/min) to the reaction vessel (+5°) containing 10 ml of 0.5 M phosphate buffer, pH 7.8, under conditions of constant air flow.

Air flow constant. The rate of removal of CO_2 by the air stream is proportional to the concentration of CO_2 in the reaction solution.[5] The proportionality constant k_f, which will be called the flow constant, includes the diffusion constant of CO_2, constants for bubble size and distribution, turbulence of liquid and air, and volume of the reaction vessel and collecting tubing. The flow constant was determined as described by Palmer,[5] and was found to be almost linearly related to the air flow rate (see Table 1). With the apparatus used in the present work k_f was found to be approximately equal to 1.0 sec^{-1} at an air flow rate of 11 ml/sec. This flow rate was used throughout the investigation.

Table 1. Relationship between air flow constant (k_f) and air flow rate.

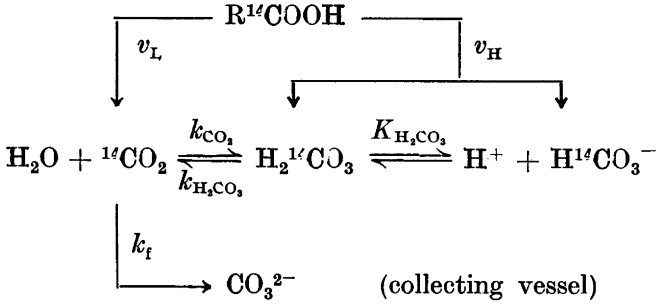
Air flow rate (ml/sec)	k_f (sec^{-1})
3.0	0.3
6.5	0.6
11.0	1.0
17.5	1.4
29.0	2.2

Orsellinic acid decarboxylase. Orsellinic acid decarboxylase was prepared from *Gliocladium roseum* mycelium as described elsewhere.[6] The enzyme preparations obtained (about 500 U/ml) were diluted with 0.5 M phosphate buffer, pH 7.8, to a final concentration of about 1 U/ml; 100 μl of this stock solution were used for each experiment. The enzyme activity was determined by manometric assays at pH 7.8, using the technique previously described.[6] Orsellinic acid decarboxylase is active over a fairly broad range of pH-values and experiments could be carried out at pH 7.8. The substrate concentrations used were adequate to saturate the enzyme during the entire reaction time, for which reason the rate of enzymatic decarboxylation could be assumed to be constant throughout the experiments.

Carboxyl-^{14}C-labelled orsellinic acid. The preparation of this substance has been described previously.[7] The specific radioactivity of the sample used in the present work was 0.28 μC/mg.

THEORETICAL

We consider the general case where an enzyme functioning both as a lyase and as a hydrolase carries out the decarboxylation of a labelled carboxylic acid $R^{14}COOH$. The scheme below indicates the events which may take place



in the reaction vessel. v_L is the rate of enzymatic formation of $^{14}CO_2$ (lyase action), and v_H the rate of enzymatic formation of $H_2{}^{14}CO_3$ and/or $H^{14}CO_3^-$ (hydrolase action). The latter two products are instantly interconverted, and $K_{H_2CO_3}$ is the first dissociation constant of H_2CO_3:

$$K_{H_2CO_3} = \frac{[H^+]\,[H^{14}CO_3^-]}{[H_2{}^{14}CO_3]} \tag{1}$$

k_{CO_2} and $k_{H_2CO_3}$ are the velocity constants for the hydration of CO_2 and the dehydration of H_2CO_3, respectively, and k_f is the flow constant (defined as described in the experimental section). The total decarboxylation rate v is equal to the sum of v_L and v_H.

The rate of change in the concentration of $^{14}CO_2$ in the reaction solution may be written as (*cf.* the above reaction scheme):

$$\frac{d[^{14}CO_2]}{dt} = v_L - (k_f + k_{CO_2})\,[^{14}CO_2] + k_{H_2CO_3}\,[H_2{}^{14}CO_3] \tag{2}$$

Since $H_2{}^{14}CO_3$ and $H^{14}CO_3^-$ are instantly interconverted we may write the sum of the rates of change of their concentration as:

$$\frac{d[H^{14}CO_3^-]}{dt} + \frac{d[H_2{}^{14}CO_3]}{dt} = v_H + k_{CO_2}\,[^{14}CO_2] - k_{H_2CO_3}\,[H_2{}^{14}CO_3] \tag{3}$$

The pH of the reaction solution can be assumed to be maintained by the buffer, and derivation of eqn. (1) with respect to time (t) then yields:

$$\frac{d[H^{14}CO_3^-]}{dt} = \frac{K_{H_2CO_3}}{[H^+]} \cdot \frac{d[H_2{}^{14}CO_3]}{dt} \tag{4}$$

Inserting this into eqn. (3) we obtain:

$$m \cdot \frac{d[H_2{}^{14}CO_3]}{dt} = v_H + k_{CO_2}[^{14}CO_2] - k_{H_2CO_3}\,[H_2{}^{14}CO_3] \tag{5}$$

where
$$m = 1 + \frac{K_{H_2CO_3}}{[H^+]} \tag{6}$$

Assuming that $[^{14}CO_2] = [H_2CO_3] = 0$ for $t = 0$ solvation of the two simultaneous first-order differential eqns. (2) and (5) yields:

$$[^{14}CO_2] = \frac{v_L + v_H}{k_f} - \frac{(r_2 - k_f)v_L + r_2 v_H}{k_f\,(r_2 - r_1)} \cdot e^{-r_1 t} - \frac{(k_f - r_1)v_L - r_1 v_H}{k_f(r_2 - r_1)} \cdot e^{-r_2 t} \tag{7}$$

where r_1 and r_2 are the two roots ($r_1 < r_2$) of the equation:

$$r^2 - (k_f + k_{CO_2} + \frac{k_{H_2CO_3}}{m})\, r + k_f \cdot \frac{k_{H_2CO_3}}{m} = 0 \tag{8}$$

The amount M of $^{14}CO_2$ removed by the air flow is obtained by integration:

$$M = \int_0^t k_f\, [^{14}CO_2]\, dt \tag{9}$$

Using the expression for $[^{14}CO_2]$ given by eqn. (7) and assuming that $[^{14}CO_2] = 0$ for $t = 0$ evaluation of eqn. (9) yields

$$M = M_L + M_H \tag{10}$$

where

$$M_L = v_L \left\{ t - \frac{r_2 - k_f}{r_2 - r_1} \cdot \frac{1 - e^{-r_1 t}}{r_1} - \frac{k_f - r_1}{r_2 - r_1} \cdot \frac{1 - e^{-r_2 t}}{r_2} \right\} \tag{11}$$

$$M_H = v_H \left\{ t - \frac{r_2}{r_2 - r_1} \cdot \frac{1 - e^{-r_1 t}}{r_1} + \frac{r_1}{r_2 - r_1} \cdot \frac{1 - e^{-r_2 t}}{r_2} \right\} \tag{12}$$

The system CO_2—H_2CO_3—HCO_3^- has been extensively and accurately studied. Rate and equilibrium constants are given and methods described in a recent review by Kern,[8] from which the values used in the present work (Tables 2 and 3) have been taken. The theoretical value of M can thus be

Table 2. Rate and equilibrium constants used in the present work. The figures have been taken from the review by Kern.[8]

Constant	Temperature 0°	5°	10°	15°	20°	25°
k_{CO_2} (sec^{-1})	0.002	0.004	0.007	0.011	0.017	0.025
$k_{H_2CO_3}$ (sec^{-1})	1.8	3.1	5.4	9.1	15.0	24.4
$K_{H_2CO_3}$	1.8×10^{-4}					

precisely predicted under any circumstances using eqns. (8) and (10)—(12). It will now be shown how experimental determinations of M as a function of time may be used to evaluate the two parameters v_L and v_H.

For $t > 10/r_2$ the term $\exp(-r_2 t)$ in eqns. (11) and (12) may be dropped. For $t < 0.05/r_1$ the term $\exp(-r_1 t) = \sum_{n=0}^{\infty} (-r_1 t)^n (n!)^{-1}$ may be expanded with retention of the first two terms in eqn. (11), and with retention of three terms in eqn. (12). Within the above interval in time we then obtain:

$$M_L = v_L \frac{k_f - r_1}{r_2 - r_1} \cdot \left(t - \frac{1}{r_2} \right) \tag{13}$$

$$M_H = v_H \frac{r_1}{r_2 - r_1} \cdot \left(\frac{r_2}{2} t^2 - t + \frac{1}{r_2} \right) \tag{14}$$

The technique used depends upon the rapid removal of CO_2 from the reaction solution, and k_f should be chosen as large as possible. At sufficient high air flow rates $[k_f \gg k_{CO_2} + (k_{H_2CO_3}/m)]$ eqn. (8) has the following approximate solution: $r_1 \approx k_{H_2CO_3}/m$; $r_2 \approx r_2 - r_1 \approx k_f - r_1 \approx k_f$. Using these values eqns. (13) and (14) may be further simplified for $t \gg k_f^{-1}$:

$$M_L = v_L t \tag{15}$$

$$M_H = v_H \frac{k_{H_2CO_3}}{2m} t^2 \tag{16}$$

Eqn. (10) may thus be re-written as:

$$\frac{M}{t} = v_L + v_H \frac{k_{H_2CO_3}}{2m} t \tag{17}$$

Eqns. (15)—(17) are approximately valid for $k_f \geq 1$ sec^{-1} and within the following interval in time:

$$\frac{50}{k_f} < t < \frac{0.2\,m}{k_{H_2CO_3}} \tag{18}$$

The air flow rate can conveniently be adjusted to fulfil the condition $k_f = 1$ sec^{-1} (*cf.* experimental section). Other experimental variables such as temperature and pH should, for practical reasons, be chosen so that the term $0.2m/k_{H_2CO_3}$ in (18) becomes larger than 5 min. Experimental determinations of M as a function of time(t) may then be used to determine v_L and v_H; according to eqn. (17) a plot of M/t against t within the interval defined by (18) will yield a straight line with the slope $v_H k_{H_2CO_3}/2m$, and intersecting the M/t-axis at v_L.

Since decarboxylases generally may be expected to function either exclusively as lyases or exclusively as hydrolases, the most common problem would be to distinguish between these two cases. For a lyase reaction we may put $v_H = 0$, $v_L = v$, and $M = M_H$ and eqn. (17) yields:

$$\frac{M}{vt} = \frac{M_L}{v_L t} = 1 \tag{19}$$

For a hydrolase reaction we similarly obtain:

$$\frac{M}{vt} = \frac{M_H}{v_H t} = \frac{k_{H_2CO_3}}{2m} t \tag{20}$$

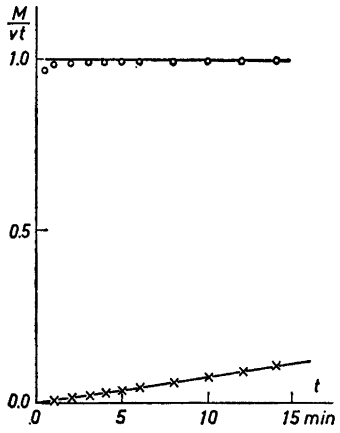


Fig. 1. Plot of M/*vt* against *t* for the lyase case (○) and the hydrolase case (×), respectively. The lines have been drawn in accordance with eqns. (19) and (20), the points have been calculated from eqns. (11) and (12), respectively. Conditions: k_f = 1 sec^{-1}; pH 7.8; + 5°; r_1 = 0.000272 sec^{-1}; r_2 = 1.004 sec^{-1}. The time interval (18) becomes 1—12 min (*cf.* Fig. 5).

As seen from Fig. 1 (which also shows the validity of the approximations) the corresponding curves are entirely different, and the above cases can readily be distinguished in a plot of M/*vt* against *t* by graphical inspection or by statistical analysis using the arithmetic mean and the regression coefficient as criteria. It may be observed that all experimental determinations of M/*vt* should be about unity for a lyase reaction, whereas M/*vt* should not exceed 0.1 within the time interval defined by (18) in the hydrolase case.

M, the total amount of collected $^{14}CO_2$, can conveniently be determined in some radioactivity measure (*e.g.* dpm). The decarboxylation rates v_L and v_H are then obtained in the corresponding measure (dpm/time unit). The total decarboxylation rate v can be obtained in the same measure by elementary calculation if the specific radioactivity of the labelled substrate and the enzyme activity (*e.g.* as determined by manometric assays) are known.

RESULTS

A typical result of the experiments in which the action of a hydrolytic decarboxylase was simulated by slow constant addition of $NaH^{14}CO_3$ to the reaction vessel is shown in Fig. 2. It is seen that the total activity of the $^{14}CO_2$ removed by the air stream increases non-linearly with time; the curve obtained is entirely different from the curve defined by eqn. (15), representing the case of immediate CO_2 production. A plot of M/*vt* against *t* (Fig. 4A) within the time interval 1—12 min yields a straight line which closely agrees with the theoretical line determined by eqn. (20). The regression coefficient obtained was 0.0080 min^{-1}, not significantly different from the theoretical value 0.0083 min^{-1}.

A typical result of the experiments with orsellinic acid decarboxylase is shown in Fig. 3. In this case the total activity of the collected $^{14}CO_2$ increases linearly with time throughout the experiment (in fact for more than 1 h) at a rate (11 800 dpm/min) which excellently agrees with the total decar-

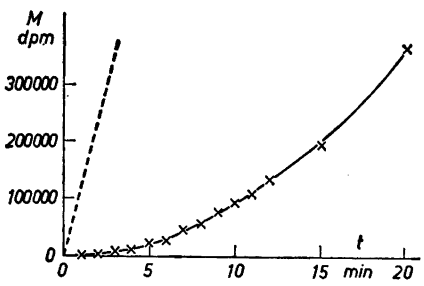


Fig. 2. Results of a typical experiment with $NaH^{14}CO_3$ (×). The dashed line shows the theoretical curve for a lyase reaction, calculated from eqn. (15). Conditions: k_f = 1 sec^{-1}; pH 7.8; +5°; v = 123 000 dpm/min.

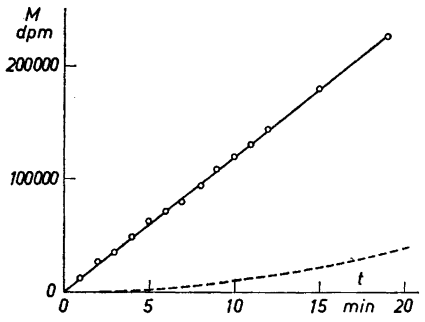


Fig. 3. Results of a typical experiment with orsellinic acid decarboxylase (○). The dashed line shows the theoretical curve for a hydrolase reaction, calculated from eqn. (16). Conditions: k_f = 1 sec^{-1}; pH 7.8; +5°; v = 11 800 dpm/min.

boxylation rate as determined by manometric assays (0.12 enzyme units; 12 500 dpm/min). In a plot of M/vt against t (Fig. 4B) all experimental points fall well along the line M/vt = 1. Statistical analysis of the data in the latter plot showed that the arithmetic mean was not significantly different from unity, and the regression coefficient not significantly different from zero. The behaviour of orsellinic acid decarboxylase thus appears to be governed by eqns. (15) and (19), and it may be concluded that orsellinic acid decarboxylase functions as a lyase, yielding CO_2 as the immediate enzymatic reaction product.

DISCUSSION

In conformity with the theoretical treatment results show that it is possible to differentiate the immediate end products of a decarboxylation reaction by the technique described in the present paper. The theoretical treatment is based upon the assumption that the interconversion of CO_2 and H_2CO_3 only involves the direct (de)hydration equilibrium (21).

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \qquad (21)$$

This equilibrium cannot be well studied except in slightly basic buffers, which favour the hydration of CO_2 while still suppressing the hydroxyl ion mechanism (22) by which CO_2 and H_2CO_3 also may be interconverted.

$$CO_2 + OH^- \rightleftharpoons HCO_3^- \qquad (22)$$

The latter mechanism competes with (21) above pH 8, being the dominating one in more alkaline solutions.[8] The technique described in the present work can, for these reasons, only be used within a fairly restricted range of pH-values (pH 7—8).

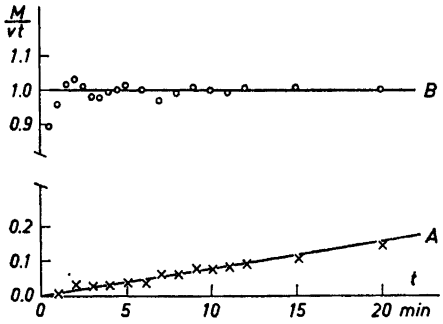


Fig. 4. Conditions as in Figs. 2 and 3, respectively.
A. Plot of M/*vt* against *t* for the experiment with $NaH^{14}CO_3$ (×), compared with the theoretical curve for a hydrolase reaction (line A) calculated from eqn. (20).
B. Plot of M/*vt* against *t* for the experiment with orsellinic acid decarboxylase (○), compared with the theoretical curve for a lyase reaction (line B) calculated from eqn. (19).

It is for many reasons preferable to carry out the experiments at a low temperature (0—5°). All rate constants for the reactions involved decrease with the temperature, thus increasing the time interval (18) during which adequate experimental data can be collected. Furthermore, the rate of the decarboxylation process increases relative to that of the non-enzymatic reactions (the activation energy for enzymatic reactions is generally low), which may be of importance when weak enzyme preparations have to be used. Similarly, the competing mechanism (22), while negligible at 0°, assumes a greater relative importance at higher temperatures, amounting to 40 % of the total at 38° even at pH 7.5.[8] Keeping the temperature low may also prevent a gradual inactivation of the enzyme preparation; in the theoretical treatment the enzyme activity is assumed to be constant throughout the experiment.

At any fixed temperature the choice of an appropriate pH-value must be made in order to obtain optimal conditions with regard to the time interval (18) during which adequate observations can be made, and to the pH-dependence of the enzyme activity and/or stability. The relationships between temperature, pH, and the upper limit of the time interval (18) are given

Table 3. The table shows the value of $0.2\ m/k_{H,CO_3}$ in minutes (the upper limit of time interval (18)) for different combinations of pH and temperature.

pH	Temperature 0°	5°	10°	15°	20°
7.0	3.3	1.9	1.1	0.7	0.4
7.1	4.2	2.4	1.4	0.8	0.5
7.2	5.3	3.0	1.8	1.1	0.6
7.3	6.7	3.8	2.2	1.3	0.8
7.4	8.4	4.8	2.8	1.7	1.0
7.5	10.6	6.0	3.5	2.1	1.3
7.6	13.3	7.6	4.4	2.6	1.6
7.7	16.8	9.6	5.6	3.3	2.0
7.8	21.1	12.1	7.0	4.2	2.5
7.9	26.6	15.2	8.9	5.3	3.2
8.0	33.4	19.1	11.1	6.6	4.0

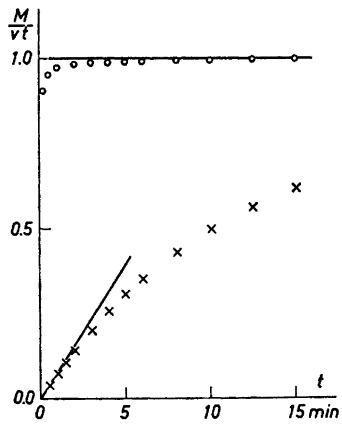


Fig. 5. Plot of M/*vt* against *t* for the lyase case (O) and the hydrolase case (×), respectively. The lines have been drawn in accordance with eqns. (19) and (20); the points have been calculated from eqns. (11) and (12), respectively. Conditions: k_f = 1 sec^{-1}; pH 7.5; +20°; r_1 = 0.0026 sec^{-1}; r_2 = 1.017 sec^{-1}. The time interval (18) becomes 1.0—1.3 min (*cf.* Fig. 1). Despite the short length of interval (18) the two cases may be well distinguished by graphical inspection.

in Table 3. It must be emphasized, however, that (18) only represents the time interval during which the approximations leading to eqns. (15) and (16) are valid (eqn. (15) in fact deviates very little from eqn. (11) as soon as the time exceeds $50/k_f$, *i.e.* after 1 min), and that the curves defined by eqns. (11) and (12) are entirely different for a much longer period. Consequently, the technique described may give valuable information also when experimental variables have to be chosen so that the time interval (18) becomes very short (see Fig. 5).

Only a few decarboxylases have been investigated with respect to the nature of the immediate reaction products, probably since the techniques previously used have been fairly complicated and of limited applicability. The case of pyruvate decarboxylase, which requires thiamine pyrophosphate as a cofactor, has been much studied, with contradictory results;[4,9,10] the latest investigation shows that CO_2 is first formed.[5] Similarly, there is evidence that a number of aminoacid decarboxylases depending on pyridoxal phosphate function as lyases.[11] There has, however, not been any evidence as to the mechanism of the action of the large group of decarboxylases that do not appear to require any cofactor.[1] The results of the present investigation clearly show that orsellinic acid decarboxylase yields CO_2 as the primary enzymatic product, providing the first experimental demonstration that an enzyme of the latter group of decarboxylases functions as a carboxy-lyase.

The technique described in the present work seems to be very simple and of general applicability. It can be used to study enzymatic as well as non-enzymatic decarboxylation reactions, it does not require the involvement of other enzyme systems such as carbonic anhydrase, and all information needed may be obtained from one single experiment. Due to the great efficiency of instruments for radioactivity measurements and to the ease with which carboxyl labelled substrates with a high specific radioactivity generally can be prepared, the technique is most sensitive and does not require powerful decarboxylase preparations. The use of carboxyl labelled substrates also makes it possible to carry out the experiments in the presence of non-radioactive

CO_2 or HCO_3^-. The technique can thus be applied also to the study of crude enzyme preparations or other complex systems carrying out decarboxylation reactions at a very slow rate.

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