

Kinetics of Synthesis of d_4 -Succinic Anhydride from Succinic Anhydride and d_1 -Acetic Acid

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Abstract □ The synthesis and the kinetics of the synthesis of d_4 -succinic anhydride from succinic anhydride and d_1 -acetic acid, catalyzed by sodium acetate, are presented. The results show that the conversion is both base and solvent catalyzed, suggesting that the deuteration may occur through the enolization of succinic anhydride. The synthesis is faster and simpler than previous methods and makes use of relatively accessible and inexpensive materials.

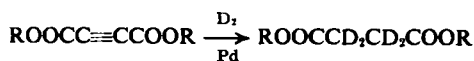
Keyphrases □ d_4 -Succinic anhydride—synthesis and kinetics from succinic anhydride and d_1 -acetic acid □ Deuteration of succinic anhydride—synthesis of d_4 -succinic anhydride □ d_1 -Acetic acid—deuteration of succinic anhydride, kinetics

The use of isotopically labeled pharmaceutical compounds as analytical tools and the selective deuteration at various points in drug molecules to investigate drug activity kinetic isotope and stability effects have been achieving wider coverage in recent years. The need to find a fast inexpensive synthesis of d_4 -succinic anhydride and deuterated succinic acid is exemplified by deuterated succinic acid's use as an important carbon source for the growth of algae, fungi, and bacteria in deuterium oxide (1-3). Growth of these organisms in deuterium oxide is severely hindered if carbon source substrates are not present. The use of these culture techniques employing deuterated and nondeuterated substrates has led to the biosynthesis of fully deuterated penicillin (4, 5), deuterated griseofulvin (6-8), deuterated clavine alkaloids (9, 10), and, more recently, L-deuterophenylalanine (11). On the basis of these needs and in the course of preparation of a number of d_4 -succinyl derivatives of drug molecules for a later study, it was indeed advisable to find a high yielding, fast synthesis of d_4 -succinic anhydride¹. Previous methods of preparation of d_4 -succinic acid and subsequently d_4 -succinic anhydride

involved the catalytic reduction of acetylenedicarboxylic acid derivatives (12-19) (Schemes I and II), the reaction of disodium succinate with alkali deuterium oxide under vigorous conditions (20-23) (Scheme III), or an enzymatic synthesis (24). All of these procedures have problems either in the preparation of starting materials (Schemes I and II and the enzymatic synthesis) or in the very vigorous conditions required for complete reaction (Scheme III). Attempts at Scheme III in these laboratories under reflux conditions proved extremely slow and costly, both in time and glassware.

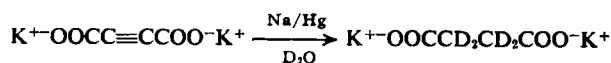
Stella and Higuchi² attempted the preparation of the *N*-succinyl derivative of phthalimide by the reaction of potassium phthalimide with succinic anhydride in nonaqueous aprotic solvents. The reaction products suggested that, under such conditions, succinic anhydride may undergo an enolization, although this was not fully investigated at the time. The partial acidic nature of the α -hydrogens of carboxylic acids (20-23, 25-30), carboxylic acid amides (23), carboxylic acid esters (31, 32) in the Stobbe reaction, and carboxylic acid anhydrides (32) in the Perkin condensation reaction has been well established. This acidic nature of the α -protons and the possible enolization of succinic anhydride prompted the present line of investigation.

In this study the synthesis and kinetics of synthesis of d_4 -succinic anhydride from succinic anhydride and d_1 -acetic acid in the presence of sodium acetate were evaluated (Scheme IV). This system fulfilled the requirements of a nonaqueous solution, a base, and a deuterium source. Haddadin (34) showed that succinic anhydride, in the presence of acetic acid at room temperature, results in the retention of the anhydride character by the succinate, with only one part in a hundred of the succinic anhydride being converted to succinic acid and acetic anhydride.

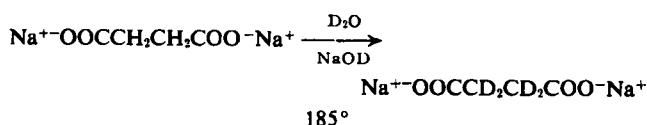


R = alkyl

Scheme I



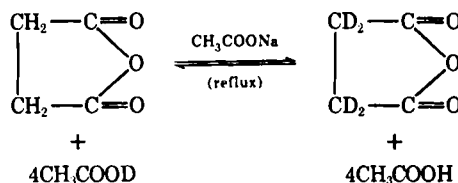
Scheme II



Scheme III

EXPERIMENTAL

Materials—Succinic anhydride was prepared by refluxing succinic acid in acetic anhydride, allowing the succinic anhydride to crystallize, and subsequently washing and recrystallizing from chloroform. d_1 -Acetic acid was prepared by stirring equimolar quantities of 99.6% deuterium oxide and acetic anhydride at 60°



Scheme IV

¹ d_4 -Succinic acid or d_4 -succinic acid can be prepared from d_1 -succinic anhydride by hydrolysis from water or deuterium oxide, respectively.

² V. J. Stella and T. Higuchi, University of Kansas, Lawrence, KS 66044, unpublished work.

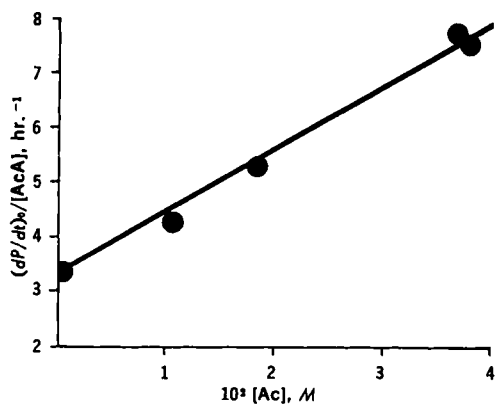


Figure 1—Plot against acetate concentration of the initial rate of formation of acetic acid carboxyl protons divided by acetic anhydride concentration for the reaction of 0.516 M acetic anhydride in d_1 -acetic acid in the presence of varying amounts of acetate ions at $99.8 \pm 0.2^\circ$.

until the reaction was complete. Reagent grade anhydrous sodium acetate, dried in a hot air oven for 24 hr., and reagent grade benzene were also used.

Kinetics—Reaction solutions were prepared by adding an appropriately weighed amount of each ingredient of the reaction mixture to a 10-ml. volumetric flask and making up to volume with d_1 -acetic acid. The solutions were transferred to 2-ml. hard-glass ampuls, flushed with nitrogen, sealed, and placed in a mineral oil bath at $99.8 \pm 0.2^\circ$. Ampuls were removed at appropriate times, the reaction was quenched by cooling in ice water, and their contents were analyzed by NMR.³

The reactions involving the rearrangement of CH_2COOD to CDH_2COOH were followed by integrating the increase of acetic acid carboxyl proton signal relative to a reference material of known concentration. The reference signal in all reactions was benzene, in a concentration of 200 mg./10 ml. The reactions involving the deuteration of succinic anhydride were followed by comparing the loss of α -proton signal of succinic anhydride relative to the internal reference signal. The results and subsequent rate constants will be expressed as succinic anhydride giving d_1 -succinic anhydride, even though the overall reaction scheme would be succinic anhydride giving d_1 -succinic anhydride which, in turn, gives d_2 -succinic anhydride, etc.

Synthesis of d_1 -Succinic Anhydride—Eight grams (0.0800 mole) of succinic anhydride and 350 mg. (0.0043 mole) of sodium acetate were refluxed in 40 ml. of d_1 -acetic acid. After 2 hr. the solution was flash evaporated, replaced with another 40 ml. of fresh d_1 -acetic acid, and again refluxed. This procedure was repeated for a total of six times. After the last reflux and on cooling, 3 g. of d_1 -succinic anhydride, m.p. $118\text{--}120^\circ$, crystallized out of solution. NMR suggested the product to be better than 97% deuterated. The filtrate was evaporated down to 10 ml., and 5 ml. of acetic anhydride was added. The suspension was heated to effect solution and allowed to cool, yielding another 3 g. of product, m.p. $119\text{--}120^\circ$. The solution was further concentrated to give 1 g. of product, m.p. $110\text{--}116^\circ$. The deuterated product, on recrystallization from anhydrous chloroform, gave m.p. $119\text{--}121^\circ$. The percentage of deuteration was assessed by dissolving 104 mg. of d_1 -succinic anhydride in a 2% sodium carbonate-deuterium oxide solution by warming. The procedure was repeated for 100 mg. of succinic anhydride in the same volume of solution. The NMR spectra of the two solutions were then compared, and the proton peak from HDO was used as the internal standard to estimate the percentage of deuteration. The results showed the d_1 -succinic anhydride to be better than 97% deuterated.

RESULTS AND DISCUSSION

In an initial attempt to synthesize d_1 -succinic anhydride via Scheme IV, the amount of acetic acid carboxyl proton formed did not correspond stoichiometrically with the amount of protons lost

Table I—Comparison of k_1 and k_2 for Succinic Anhydride versus Those for Acetic Anhydride

Anhydride	k_1 , hr. ⁻¹	k_2 , M ⁻¹ hr. ⁻¹
Acetic anhydride	3.35×10^{-3}	1.15
Acetic anhydride*	2.23×10^{-3}	7.70×10^{-1}
Succinic anhydride	9.75×10^{-3}	2.37

* Corrected for the fact that there are six exchangeable protons in acetic anhydride relative to four in succinic anhydride.

from the isolated, partially deuterated succinic anhydride. It was postulated that the excess protons had come from acetic anhydride by incorporation of deuterium into the methyl groups of acetic anhydride and, subsequently, acetic acid; i.e., acetic anhydride present in the solution was competing with the succinic anhydride for the deuterium. The acetic anhydride resulted from: (a) the reaction of succinic anhydride with acetic acid to form succinic acid and acetic anhydride (34), (b) excess acetic anhydride from the production of d_1 -acetic acid, or (c) the fact that, under reflux conditions, acetic anhydride (and the corresponding amount of water) is formed in low concentration from acetic acid (35–37).

To check if this side reaction could be controlled, a kinetic investigation was conducted to observe the incorporation of deuterium into the methyl groups of acetic anhydride and, subsequently, acetic acid from d_1 -acetic acid. The rate of production of carboxyl proton, dP/dt , at 99.8° was followed as a function of sodium acetate concentration, [Ac], and acetic anhydride concentration, [AcA]. The curves obtained from the reaction, complete to equilibrium, followed neither strict first- nor second-order kinetics. The results, therefore, are presented in terms of initial rates. The results suggest that the rate of production of carboxyl protons follows Eq. 1:

$$\left(\frac{dP}{dt}\right)_0 = k_1[\text{AcA}] + k_2[\text{AcA}][\text{Ac}] \quad (\text{Eq. 1})$$

where $(dP/dt)_0$ is the initial rate of production of carboxyl protons in molar concentration per hour, k_1 is the solvent-catalyzed rate constant, and k_2 is the acetate-catalyzed rate constant.

The rate constants k_1 and k_2 are pseudo-first-order constants in that they assume the d_1 -acetic acid concentration to remain relatively constant in the initial time period. Equation 1 predicts that a plot of $(dP/dt)_0/[\text{AcA}]$ versus [Ac] would give a straight line of slope k_2 and intercept k_1 (Fig. 1). This gave values for k_2 of $1.15 \text{ M}^{-1} \text{ hr.}^{-1}$ and k_1 of $3.35 \times 10^{-3} \text{ hr.}^{-1}$. Eq. 1 also predicts that a plot of $(dP/dt)_0$ versus [AcA] for a given acetate concentration would give a straight line of slope equal to $k_1 + k_2[\text{Ac}]$ and intercept of zero (Fig. 2). The least-squares slope of this line is $7.88 \times 10^{-3} \text{ hr.}^{-1}$. The slope of the line calculated using the data obtained from Fig. 1 and the corresponding acetate concentration would have predicted a slope of $7.60 \times 10^{-3} \text{ hr.}^{-1}$. It does appear, therefore, that under the conditions of the experiment and the limited accuracy of the NMR

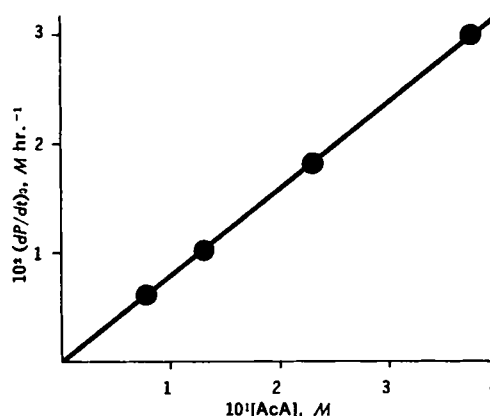


Figure 2—Plot against acetic anhydride concentration of the initial rate of formation of acetic acid carboxyl protons for the reaction of acetic anhydride in d_1 -acetic acid at $99.8 \pm 0.2^\circ$ in the presence of $3.69 \times 10^{-2} \text{ M}$ acetate ions.

³ Varian A60.

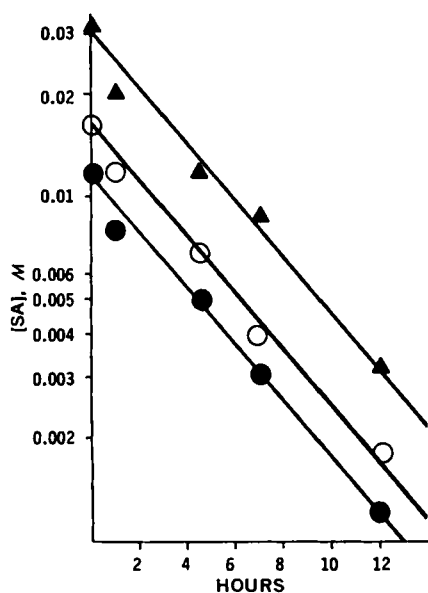


Figure 3—Plot against time of logarithms of residual concentration of succinic anhydride during its deuteration at $99.8 \pm 0.2^\circ$ in d_1 -acetic acid containing 3.64×10^{-2} M sodium acetate as a catalyst. Initial succinic anhydride concentrations were 3.16×10^{-2} M (\blacktriangle), 1.60×10^{-2} M (\circ), and 1.12×10^{-2} M (\bullet).

technique, Eq. 1 does adequately describe the production of the carboxyl protons.

The formation of d_1 -succinic anhydride was followed by observing directly the loss of succinic anhydride protons. Because of the conditions used, *i.e.*, an excess of d_1 -acetic acid, the loss of succinic anhydride protons followed pseudo-first-order kinetics. The pseudo-first-order rate constants for the disappearance of the α -protons of succinic anhydride were independent of succinic anhydride concentration, [SA] (Fig. 3). The dependency on acetate concentration is seen in Figs. 4 and 5. The results appeared to follow the same kinetics as for acetic anhydride and were described by Eq. 2:

$$-\frac{dP'}{dt} = k_1[SA] + k_2[SA][Ac] \quad (\text{Eq. 2})$$

where $-dP'/dt$ is the rate of loss of succinic anhydride protons in molar concentration per hour.

From Figs. 4 and 5, k_2 was found to equal $2.37 M^{-1} \text{hr}^{-1}$ and k_1 to equal $9.75 \times 10^{-2} \text{hr}^{-1}$. The rate of carboxyl proton formation again could not be totally accounted for on the basis of what had been formed from the succinic anhydride. However, in light of the results presented earlier in this discussion, the small amount of extra protons could be accounted for in terms of the analogous reaction of acetate and solvent reacting with the small amounts of acetic anhydride present in solution.

As described by Eqs. 1 and 2, the deuterium incorporation in acetic anhydride (and, subsequently, acetic acid) and succinic anhydride is both base and solvent catalyzed. The incorporation of deuterium *via* enolization into other carbonyl compounds such as ketones and aldehydes is both acid and base catalyzed, suggesting that a possible rate-determining transition step in the present study involves the enolization of succinic anhydride to Compound I and a similar structure for acetic anhydride. The rate dependency on acetate ions leads to the possibility of acetate catalyzing an enolization *via* a transition state of the type shown in Scheme V, while the solvent catalyzes the reaction *via* an acid-catalyzed enolization. These results do not preclude a concerted acid- and base-catalyzed pathway. On regeneration of the nonenol form of succinic anhydride, the molecule picks up a deuterium instead of a proton. This process continues until equilibrium is established.

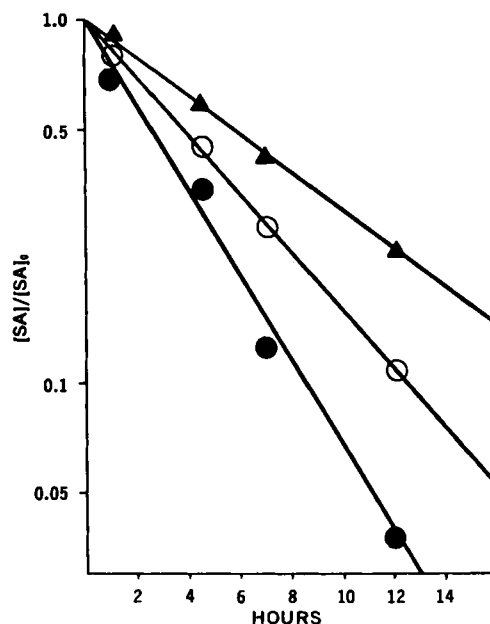
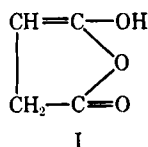


Figure 4—Plot against time of logarithms of residual/initial concentration of succinic anhydride during its deuteration at $99.8 \pm 0.2^\circ$ in d_1 -acetic acid containing 1.28×10^{-2} M (\blacktriangle), 3.64×10^{-2} M (\circ), and 7.31×10^{-2} M (\bullet) sodium acetate.

No attempts were made at this stage to determine the deuterium-hydrogen isotope effects for any steps in these reactions or the catalytic effects of other acids or bases. Work along these lines will be continued. Because anhydrides are known to undergo the Perkin condensation reaction under basic conditions by the reaction of the anhydride- α -anion at carbonyl centers, it seemed advisable to check whether products like 2-acetylsuccinic anhydride might form. Succinic anhydride was refluxed for 18 hr. in acetic acid, with sodium acetate present in approximately the same concentration as in the kinetic study. No new peaks or anomalies were seen by NMR, which would have suggested the presence of 2-acetylsuccinic anhydride or similar products.

Table I shows the values of k_1 and k_2 for succinic anhydride relative to those of acetic anhydride. These results show that succinic anhydride is more susceptible to exchange (and enolization) than acetic anhydride, a fact probably attributable to its ability to undergo delocalization of its electrons more favorably because of its planar nature. The values compared in Table I also are not a true indication of the differences between succinic anhydride and acetic anhydride exchange rates and actually minimize the difference. When the increase in carboxyl proton signal was being measured for the reaction involving the proton release from acetic anhydride, the reaction being measured was d_1 -acetic acid reacting with acetic anhydride to give acetic acid and d_1 -acetic anhydride.

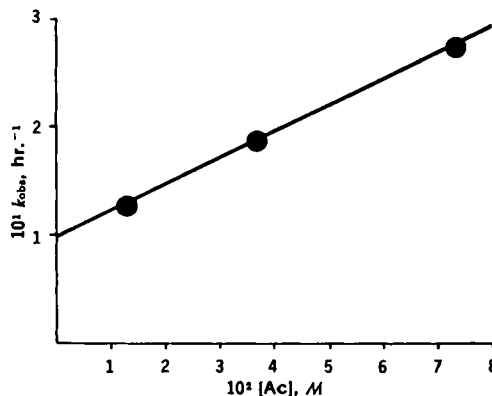
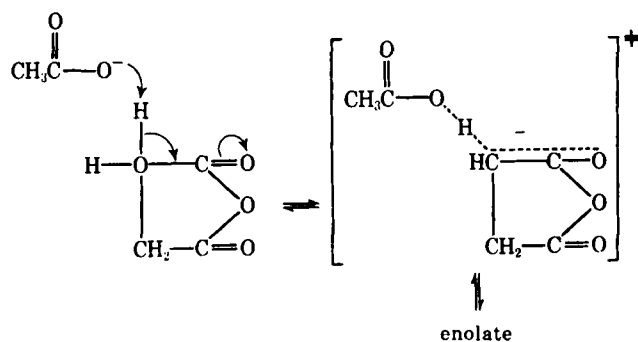


Figure 5—Plot against acetate concentration of the pseudo-first-order rate constant for the deuteration of succinic anhydride to d_1 -succinic anhydride at $99.8 \pm 0.2^\circ$ in d_1 -acetic acid.



Scheme V

However, d_1 -acetic anhydride reacts with d_1 -acetic acid or acetic acid to give d_1' -acetic acid (CDH_2COOH) or d_2 -acetic acid (CDH_2COOD) and acetic anhydride. Equilibrium is established when the concentration of $RCOOH$ approaches that of $RCOOD$. At equilibrium the acetic anhydride is present mainly as d_1 -acetic anhydride, which still has five exchangeable protons. Under the same reaction conditions, succinic anhydride would have been converted to d_1 -succinic anhydride and, subsequently, d_2 -succinic anhydride, d_3 -succinic anhydride, and d_4 -succinic anhydride. If the reaction is not stopped at this stage and acetic acid and d_1' -acetic acid are allowed to build up, d_4 -succinic anhydride is slowly converted back to d_2 -succinic anhydride, etc.

The results of the kinetic study show that succinic anhydride can be successfully deuterated in d_1 -acetic acid as long as attempts are made to ensure that the smallest possible amount of acetic anhydride is present. A synthetic scale reaction was successfully attempted and has been described. By carefully preparing d_1 -acetic acid from deuterium oxide and acetic anhydride with deuterium oxide in very slight excess and reacting this with succinic anhydride in the presence of sodium acetate, the synthesis resulted in a product that was better than 97% deuterated and a yield of 86%. The reaction uses simple chemicals and makes use of the partial acidic nature of the α -protons of succinic anhydride and the probable tendency of the molecule to enolize.

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