(7) H. R. Helfant, V. M. Herman, and R. Gorlin, *Circulation*, **43**, 641 (1971).
(8) R. S. Ross, "Myocardial Ischemia," Excerpta Medica, Amsterdam, The Netherlands, 1971, p. 98.
(9) J. T. Willerson, W. J. Powell, T. E. Guiney, J. J. Stark, C. A. Sanders, and A. Leaf, *J. Clin. Invest.*, **51**, 2989 (1972).
(10) J. B. Caulfield, J. T. Willerson, M. L. Weisfeldt, and W. J. Powell, "Myocardial Metabolism," University Park Press, Baltimore, Md., 1972, p. 753.
(11) J. T. Willerson, M. L. Weisfeldt, C. A. Sanders, and W. J. Powell, *Cardiovas. Res.*, **8**, 8 (1974).
(12) E. Braunwald and C. J. Frahm, *Circulation*, **24**, 633 (1961).
(13) J. S. Arnoff, E. Braunwald, H. G. Welch, R. B. Case, N. W. Stainsby, and R. Macruz, *Am. J. Physiol.*, **192**, 148 (1958).
(14) A. A. Walker, J. S. Janicki, K. T. Weber, R. O. Russell, and C. E. Rackley, *Cardiovasc. Res.*, **7**, 567 (1973).
(15) G. W. Snedecor and W. G. Cochran, "Statistical Methods," Iowa State University Press, Ames, Iowa, 1967, p. 59.
(16) B. A. Hill, "Principles of Medical Statistics," Oxford University Press, New York, N.Y., 1961, p. 149.
(17) J. T. Reeves, L. L. Hefner, B. W. Jones, C. Coghlan, G. Prieto, and J. Carrol, *Am. Heart J.*, **60**, 745 (1960).
(18) J. Flores, D. R. Dibona, C. H. Beck, and A. Leaf, *J. Clin. Invest.*, **51**, 118 (1972).
(19) A. Ames, R. L. Wright, M. Kowada, J. M. Thurston, and G. Majno, *Am. J. Pathol.*, **52**, 437 (1968).
(20) K. W. Summers and R. L. Jamison, *Lab. Invest.*, **25**, 635 (1971).
(21) J. Koch-Weser, *Am. J. Physiol.*, **204**, 957 (1963).
(22) C. S. Apstein, O. L. Bing, and H. J. Levine, *J. Mol. Cell. Cardiol.*, **8**, 627 (1976).
(23) J. S. Sarnoff, J. P. Gilmore, and A. G. Wallace, "Nervous Control of the Heart," Williams & Wilkins, Baltimore, Md., 1965, pp. 54–129.
(24) T. Watanabe, J. W. Covell, P. R. Maroko, E. Braunwald, and J. Ross, *Am. J. Cardiol.*, **30**, 371 (1972).
(25) R. Balcon, *Postgrad. Med. J., Suppl.*, **47**, 53 (1971).

Synthesis and Cyclization of Dialkylmalonuric Esters

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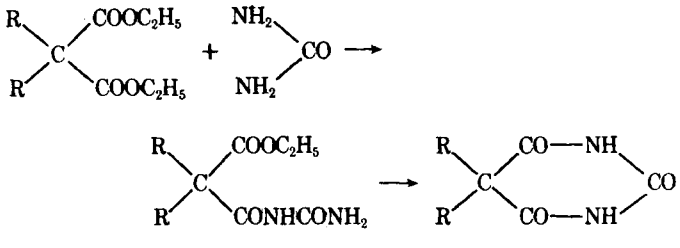
Abstract □ A novel method for the synthesis of methyl dialkylmalonuric esters was developed using the base-catalyzed ring opening of an isopropylidene malonic ester with urea as the key step. The rates of cyclization of these malonuric esters to the corresponding barbituric acids then were studied at buffer concentrations ranging from 0.01 to 1.00 *M*. The reaction was shown to be general base catalyzed, and the reaction rate was found to be subject to a deuterium isotope effect, $k_{H_2O}/k_{D_2O} = 1.3$. The thermodynamic activation parameters also were determined. A three-step mechanism for the conversion of malonuric esters to barbituric acids was proposed; it involved a rapid cyclization step, followed by proton removal by a general base catalyst and a rate-determining collapse of the resulting tetrahedral intermediate aided by a general acid.

Keyphrases □ Dialkylmalonuric esters—synthesis, cyclization to barbituric acids □ Barbituric acids—mechanism for formation by cyclization of malonuric acids □ Activation parameters—cyclization of dialkylmalonuric esters to barbituric acids

Numerous barbituric acids have been synthesized over the past 75 years, but the mechanism of the reaction has not been defined. Barbituric acids usually are prepared by reacting a malonic ester with urea in the presence of a basic catalyst. The mechanism proposed some years ago (1) involved the formation of an intermediate malonuric ester (I), followed by rapid ring closure to the barbituric acid product (Scheme I).

The fact that malonuric esters undergo facile cyclization in basic media was suggested in 1907 (2), and later these esters were shown to cyclize rapidly in weakly basic solution (1). In a continuing effort to elucidate the mechanism of the reaction between malonic esters and urea, the present study focuses on the cyclization step.



I

Scheme I

BACKGROUND

Although no kinetic studies on the cyclization of malonuric esters have been reported, hydrolysis of barbituric acids in alkaline solution has been investigated. Such hydrolyses produce various products, including the malonuric acid. A complete scheme for the alkaline decomposition of barbiturates was given by Aspelund (3), and a kinetic study on the hydrolysis rate of barbituric acid derivatives was reported by Garrett *et al.* (4). The latter report stated that malonuric acids are capable of hydrolysis to the barbituric acids at pH 7–10, and the enthalpy (ΔH^*) and entropy (ΔS^*) of activation were determined for the hydrolysis of numerous barbituric acids.

One facet of the present investigation involved determination of the mechanism of base catalysis in the cyclization of esters of diethylmalonuric acid. The classical experiment for distinguishing general base catalysis from specific base catalysis involves determining the reaction rate in a series of buffers of constant pH but with varying concentrations of total buffer species at constant ionic strength (5). General base catalysis, which requires catalysis by all bases present, is demonstrated if the reaction rate depends on the total absolute buffer concentration. Specific base catalysis is independent of the absolute buffer concentration.

UV spectrophotometry was employed to monitor the cyclization reaction of the malonuric esters. A pH 10 buffer system was chosen since at that pH the barbituric acid product exists as the monoanionic species (99+%), which absorbs strongly at 240 nm (6), facilitating easy assessment of its formation rate.

This method also was used to measure the reaction rates in deuterium oxide since a comparison of rates in heavy *versus* light water can be valuable in mechanism elucidation. Jencks (7) reported that because of zero-point energy differences and different stretching frequencies, rate constants for OH and OD bond cleavage can be 10-fold larger for hydrogen than for deuterium.

Determination of the thermodynamic activation parameters also was undertaken. The entropy and enthalpy of activation were obtained from plots of $\log k/T$ *versus* $1/T$ (8).

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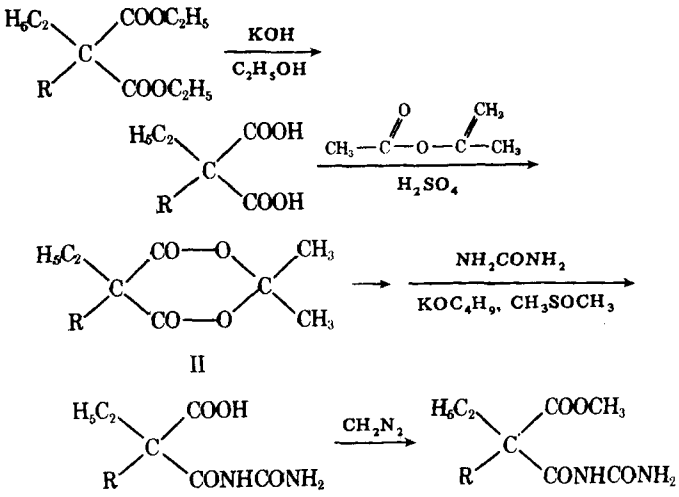
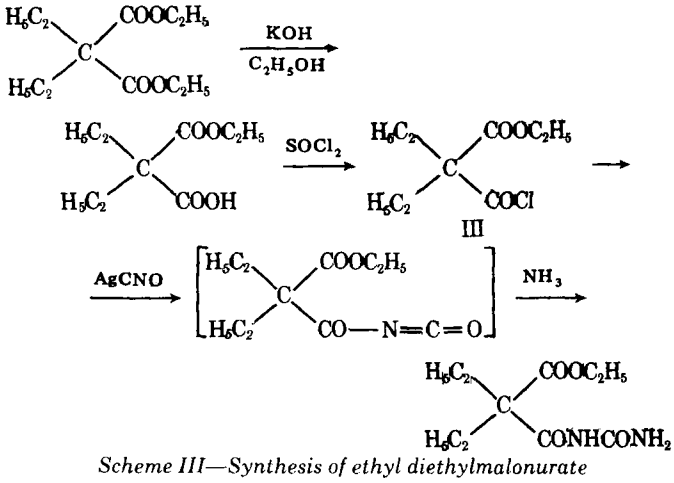


Scheme II—Synthesis of methyl esters of dialkylmalonuric acids



Scheme III—Synthesis of ethyl diethylmalonurate

EXPERIMENTAL

Synthesis of Reactants—The methyl esters of diethylmalonuric acid and ethylisoamylmalonuric acid were prepared by the sequence shown in Scheme II. This reaction represents a novel procedure for the preparation of various methyl dialkylmalonuric esters from the readily available malonic esters. The isopropylidene malonate (II), prepared by a modified method of Davidson and Bernhard (9), serves as a convenient intermediate for the base-catalyzed conversion to the malonuric acid. This reaction presumably occurs *via* a nucleophilic attack of urea on the carbonyl group of II with simultaneous ring opening and elimination of acetone.

The ethyl ester of diethylmalonuric acid was prepared by the sequence shown in Scheme III. Preparation of the intermediate diethylmalonyl chloride monoethyl ester (III) was accomplished using standard procedures, and its conversion into the malonuric ester was reported previously (1). In this instance, the acyl isocyanate was not isolated but was treated immediately upon generation with aqueous ammonia to give a mixture of the desired ethyl ester of diethylmalonuric acid and a substantial amount of the ethyl ester of diethylmalonamic acid, which was separated by fractional crystallization from ligroin.

Reagents—Ethanolamine was redistilled. Concentrated hydrochloric acid was refluxed until a constant vapor temperature of 107° was obtained, and then it was standardized as 6.36 *M* against tris(hydroxymethyl)aminomethane. Reagent grade potassium chloride, sodium bicarbonate, and sodium carbonate were used without further purification. The deuterium oxide, 99.8% pure, was used as received. Sodium deuteriocarbonate was prepared by dissolving 0.5 g of sodium bicarbonate in 10 ml of deuterium oxide and evaporating the solution to dryness (10).

Syntheses[1]—*Diethylmalonic Acid*—Diethyl diethylmalonate, 79.5 ml (0.37 mole), was added dropwise to a refluxing solution of 123 g (2.20 moles) of potassium hydroxide in 500 ml of ethanol. After the mixture was heated for 3–4 hr, a white solid began to separate. Then the mixture was cooled, and the precipitate was filtered off and washed once with cold ethanol.

The solid was purified by dissolving it in water, acidifying the solution, and collecting the crystalline free acid by filtration. The yield was 39.4 g (67%) of colorless crystals, mp 122–127° [lit. (11) mp 125°]; IR (KBr): 3440 (OH), 1700 (C=O), 1410, and 915 cm^{-1}.

Isopropylidene Diethylmalonate (II)—Sulfuric acid (0.5 ml) was added cautiously to a mixture of 10.0 g (62.5 mmoles) of diethylmalonic acid and 7.6 ml (70 mmoles) of isopropenyl acetate. Then a second equivalent (70 mmoles) of isopropenyl acetate was added, and the mixture was heated briefly to 40°. It then was stirred for 4–5 hr at room temperature. After removal of the volatiles at reduced pressure, the residue was dissolved in ether, washed with sodium bicarbonate and water, and dried over magnesium sulfate.

Removal of the ether left an orange oil. This oil was vacuum distilled to yield 2.9 g (23%) of a colorless liquid, bp 97–100° (0.3 mm), which crystallized on standing to a colorless solid, mp 35–37.5°; IR (KBr): 1785 and 1755 (C=O) cm^{-1}; NMR (acetone-d_6): δ 1.93 (q, 4H, CH_2), 1.66 [s, 6H, $C(CH_3)_2$], and 0.87 (t, 6H, CH_3).

Anal.—Calc. for $C_{10}H_{16}O_4$: C, 60.04; H, 8.06; saponification equivalent 200. Found: C, 60.16; H, 7.90; saponification equivalent 201.

Methyl Diethylmalonurate—A solution of isopropylidene diethylmalonate (2.00 g, 10 mmoles) and urea (0.90 g, 15 mmoles) in 20 ml of dry dimethyl sulfoxide was added slowly, with stirring, to a solution of 2.24 g (20 mmoles) of potassium *tert*-butoxide in 20 ml of dimethyl sulfoxide. Stirring was continued at room temperature for 4 hr. Then the mixture was poured into 100 ml of ice water and extracted once with 50 ml of ether.

The aqueous layer was acidified with hydrochloric acid and extracted three times with 50 ml of ether. These extracts were dried over magnesium sulfate, and the solvent was removed under vacuum, depositing a crystalline residue. This residue was recrystallized from water, yielding 0.47 g (25%) of diethylmalonuric acid as colorless platelets, mp 158–161° [lit. (12) mp 162°]; IR (KBr): 1710, 1650, and 1250 cm^{-1}.

An ethereal solution containing a three- to fivefold excess of diazomethane (generated from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide[2]) at 0° was added, with stirring, to an ether solution of 0.26 g (1.3 mmoles) of diethylmalonuric acid at 0°. The mixture was stirred for 1 hr at room temperature, and the ether then was evaporated under a nitrogen stream to yield an oil that solidified on cooling. The solid was recrystallized from ligroin to give 116 mg (42% yield) of colorless needles, mp 112–114.5°; IR (KBr): 3420, 1725, 1700, and 1585 cm^{-1}; NMR ($CDCl_3$): δ 3.70 (s, 3H, OCH_3), 1.90 (q, 4H, CH_2), and 0.78 (t, 6H, CH_3).

Anal.—Calc. for $C_9H_{16}N_2O_4$: C, 49.99; H, 7.46. Found: C, 50.04; H, 7.46.

Methyl Ethylisoamylmalonurate—Methyl ethylisoamylmalonurate was prepared from diethyl ethylisoamylmalonate by the same route as for methyl diethylmalonurate. It was obtained as colorless crystals from ligroin, mp 74–76° [lit. (13) mp 74–75°]; IR (KBr): 3410, 1740, 1715, 1690, and 1570 cm^{-1}; NMR ($CDCl_3$): δ 3.70 (s, 3H, OCH_3), 1.85 (m, CH_2), and 0.80 [d, 6H, $CH(CH_3)_2$].

General Buffer Preparation[3]—Buffers for the kinetic studies were prepared with ethanolamine in carbon dioxide-free distilled water and were adjusted to pH 10.0 with constant boiling hydrochloric acid. Potassium chloride was added when necessary to maintain the ionic strength. The buffers were prepared on a cold plate at the experimental temperature at concentrations of 0.011, 0.110, and 1.100 *M* with respect to ethanolamine so that, upon dilution with the malonuric ester solutions, the final concentrations for the kinetic studies were 0.010, 0.100, and 1.000 *M*, respectively.

For the deuterium oxide study, the buffers were prepared from sodium carbonate and sodium deuteriocarbonate to give a final concentration of 0.18 *M* total buffer at pH 10.0 and 15°.

General Spectrophotometric Method[4]—Malonuric ester solutions were prepared with either carbon dioxide-free distilled water or degassed deuterium oxide at 10^{-3} *M* and were used on the same day. The malonuric

[1] Melting points were determined with a Fisher Mel-Temp apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer model 457 grating spectrophotometer, and NMR spectra were recorded on a Hitachi Perkin-Elmer R-20B spectrometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn.

[2] Diazald, Aldrich Chemical Co.

[3] All pH measurements were made with a Corning model 10 pH meter equipped with a glass electrode and a potassium chloride reference electrode. The meter was standardized with buffer solutions at the experimental temperature before use.

[4] UV spectral data were obtained on a Beckman DK-2A recording spectrophotometer.

Table I—First-Order Rate Constants for the Cyclization of Diethylmalonuric Esters

Medium	k, min^{-1} [a] Methyl Diethylmalonurate	Ethyl Diethylmalonurate
0.01 *M* Buffer[b,c]	0.61	0.28
0.10 *M* Buffer[b,c]	0.79	0.38
1.00 *M* Buffer[b,c]	1.07	0.51
Water[c,d]	0.93	0.43
Deuterium oxide[c,e]	0.70	0.33

[a] The k values represent an average of at least six determinations. [b] Ethanolamine–hydrochloric acid buffer; the ionic strength was maintained at 0.51 *M* with potassium chloride. [c] Reaction temperature of 15.0 ± 0.1°. [d] Buffered with sodium carbonate–sodium bicarbonate at 0.18 *M*. [e] Buffered with sodium carbonate–sodium deuteriocarbonate at 0.18 *M*.

ester solution, the appropriate buffer, and the cell compartment of the spectrophotometer were preequilibrated at the experimental temperature (±0.1°). The reactions were initiated by injecting 0.3 ml of the malonuric ester solution from a calibrated syringe into the spectrophotometer cell already containing 2.7 ml of buffer. The solution was agitated and placed quickly into the spectrophotometer cell compartment. Absorbance at 240 nm was recorded as a function of time compared to the pure buffer solution reference.

Data for the calculation of the rate constants were obtained directly from the absorbance plots and were treated by the Guggenheim method (14).

RESULTS

The cyclization of the dialkylmalonuric esters was studied in aqueous ethanolamine–hydrochloric acid buffers at pH 10.0 over total buffer concentrations ranging from 0.01 to 1.00 *M*. The rate constants for the methyl and ethyl esters of diethylmalonuric acid are given in Table I. The rates varied with total absolute buffer concentration, consistent with a mechanism involving general base catalysis. Since the reaction is an ester aminolysis, the mechanism proposed by Bunnett and Davis (15) implicating general base catalysis in the reaction of butylamine with ethyl formate might be expected to apply. In addition, general base catalysis in the aminolysis of phenyl acetate was observed (10), and susceptibility to general base catalysis appears to be a general characteristic of ester aminolysis reactions (15).

The thermodynamic activation parameters are shown in Table II for the same two esters and the methyl ester of ethylisoamylmalonuric acid. The values of the thermodynamic parameters indicate that the variation in overall cyclization rates was due largely to the ΔS^* term. The ΔH^* values were all close (<5% difference) and actually in reverse order to the measured rates. The methyl ester, which reacted fastest, had the highest ΔH^* value. However, the ΔS^* values were significantly different and closely paralleled the observed rates. In fact, a plot of ΔS^* *versus* k for the three esters was linear. Combining the ΔH^* and ΔS^* terms produced an overall ΔG^* that paralleled the observed rate differences.

The cyclization rates in water and deuterium oxide containing sodium carbonate–bicarbonate buffers also are shown in Table I. Both esters showed a rate decrease in deuterium oxide of approximately the same magnitude, $k_{H_2O}/k_{D_2O} = 1.3$.

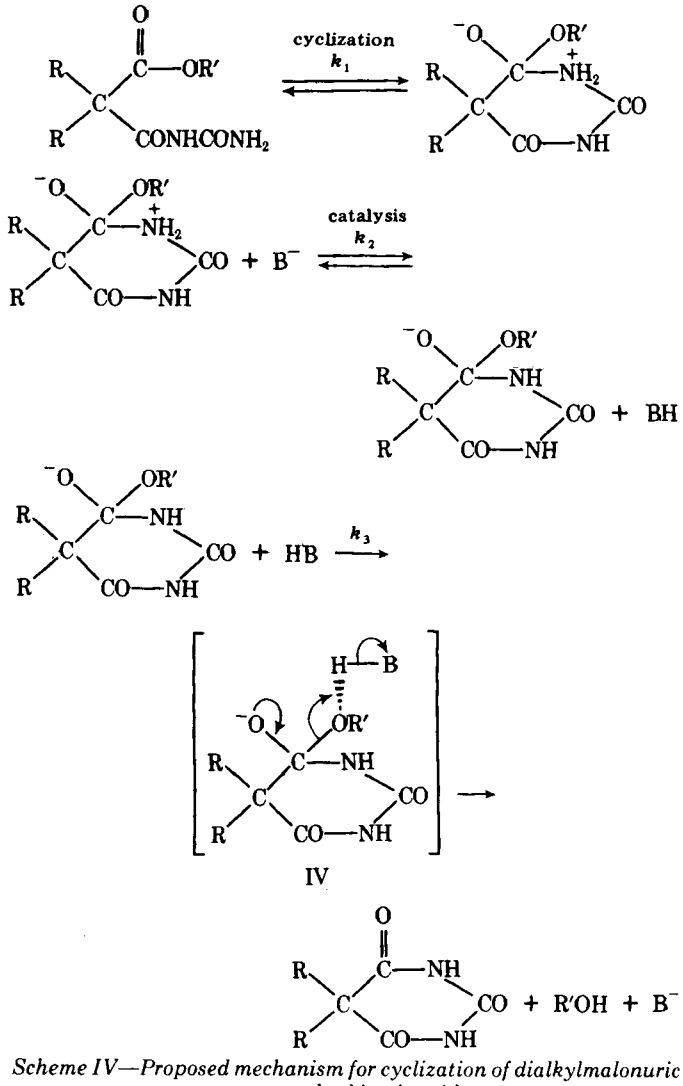
DISCUSSION

A probable mechanism for the cyclization of malonuric esters, based on the general mechanism for ester aminolysis given by Bunnett and

Table II—First-Order Rate Constants and Activation Parameters for the Cyclization of Dialkylmalonuric Esters

Compound	k, min^{-1} [a,b] 10°	15°	20°	Activation Parameters ΔH^*, kcal/mole	ΔS^*, cal/K° mole	ΔG^*, kcal/mole
Methyl diethylmalonurate	0.59	1.06	1.91	18.8	−3.2	19.7
Methyl ethylisoamylmalonurate	0.47	0.80	1.49	18.4	−5.1	19.9
Ethyl diethylmalonurate	0.29	0.51	0.89	17.9	−7.8	20.1

[a] The k values represent an average of at least six determinations. [b] The buffer concentration was 1.00 *M*, and the ionic strength was 0.59 *M*.

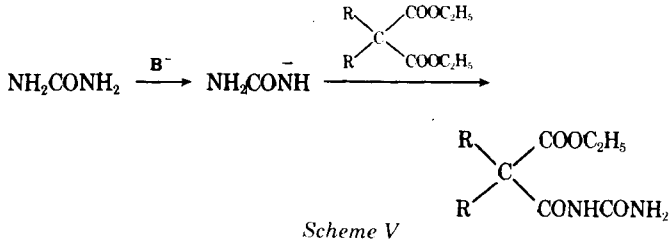


Scheme IV—Proposed mechanism for cyclization of dialkylmalonuric esters to barbituric acids

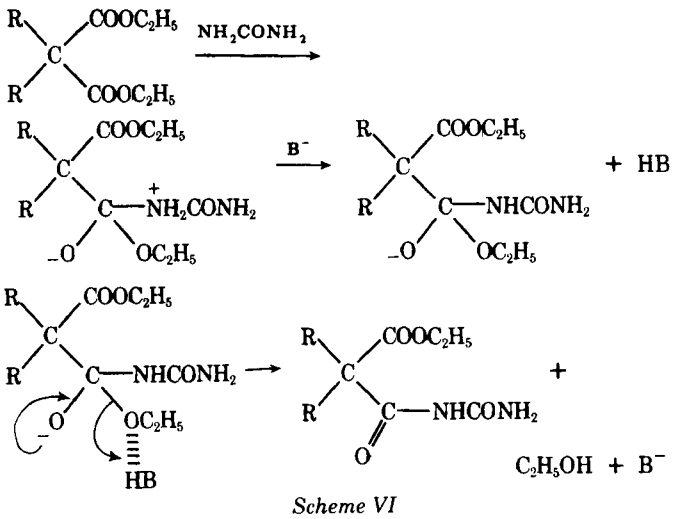
Davis (15), is shown in Scheme IV. Several kinetic pathways are possible, depending on which step is rate determining. If the cyclization step is slow (k_2 and $k_3 > k_1$), the reaction is subject to specific base catalysis since the strongest base in the medium, OH^-, should eliminate catalysis by the weaker bases once the cyclization occurs. However, if catalysis is rate limiting ($k_1 > k_2$ and k_3), then general base catalysis is observed since all basic species present should catalyze these steps at individual measurable rates.

Since the reaction rate of both the methyl and ethyl esters has been shown experimentally to decrease by a factor of 1.8 as the total buffer concentration is varied 100-fold, the reaction must be subject to general base catalysis, and the rate-determining step is either the catalytic step, k_2, or the collapse of the tetrahedral intermediate aided by the general acid (HB), k_3. The measured deuterium isotope effect, $k_H/k_D = 1.3$, tends to implicate k_3 as the limiting step. Even though the observed isotope effects are small compared to normal d^2 isotope effects, k_H/k_D values in the range of 1.1–1.5 have been reported for the general base-catalyzed reaction of phenyl acetate with glycine and ammonia (10).

The observations that substitution of an isoamyl group for an ethyl group in the acyl portion of the malonuric ester caused a rate decrease



Scheme V



Scheme VI

by a factor of 1.3 and, more importantly, that substitution of the ethyl ester for the methyl ester decreased the rate by a factor of 2.1 also are consistent with a rate-limiting collapse of the tetrahedral intermediate. This dependence on the bulkiness of both the acyl substituents and the leaving alkoxy groups can be attributed to steric hindrance from formation of the hydrogen-bonded intermediate (IV). It also seems reasonable that the identity of the alkoxy group should play the greater role since it is closer to the reaction site. On the other hand, the identity of these groups should have little effect on the proton removal in the catalytic step (k_2).

In view of these findings, it also seems reasonable that the mechanism proposed for the formation of the malonuric ester from urea and the malonic ester needs to be revised (1). Rather than the base functioning to form urea anions, which subsequently attack the malonic ester (Scheme V), the base probably functions in proton removal from the dipolar tetrahedral intermediate that results from the initial attack of neutral urea on the malonic ester (Scheme VI).

REFERENCES

(1) J. A. Beres, D. E. Pearson, and M. T. Bush, *J. Med. Chem.*, **10,** 1078 (1967).

(2) C. F. Boeringer and Sons, German pat. 193 447 (1907); through *Chem. Zentralbl.*, **1908I,** 1000.

(3) H. Aspelund, *Acta Acad. Abo., Ser. B,* **20** (1955); through *Chem. Abstr.*, **50,** 11351f (1956).

(4) E. R. Garrett, J. T. Bojarski, and G. J. Yakatan, *J. Pharm. Sci.*, **60,** 1145 (1971).

(5) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill, New York, N.Y., 1940, p. 215.

(6) M. E. Krall, *J. Phys. Chem.*, **44,** 449 (1940). J. C. Butler, J. M. Ruth, and G. F. Tucker, *J. Am. Chem. Soc.*, **77,** 1486 (1955).

(7) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N.Y., 1969, p. 247.

(8) J. A. Hirsch, "Concepts in Theoretical Organic Chemistry," Allyn and Bacon, Boston, Mass., 1974, p. 124.

(9) D. Davidson and S. A. Bernhard, *J. Am. Chem. Soc.*, **70,** 3426 (1948).

(10) W. P. Jencks and J. Carriuolo, *J. Am. Chem. Soc.*, **82,** 675 (1960). W. P. Jencks and M. Gilchrist, *ibid.*, **88,** 104 (1966).

(11) D. Heilbron and H. M. Bunbury, "Dictionary of Organic Compounds," vol. II, Eyre and Spottiswoode, London, England, 1953, p. 186.

(12) E. Fischer and A. Dilthey, *Ann.*, **335,** 334 (1904).

(13) E. W. Maynert and E. Washburn, *J. Org. Chem.*, **15,** 259 (1950).

(14) G. M. Fleck, "Chemical Reaction Mechanisms," Holt, Rinehart and Winston, New York, N.Y., 1971, p. 37.

(15) J. F. Bunnett and G. T. Davis, *J. Am. Chem. Soc.*, **82,** 665 (1960).

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