[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN CO.]

Evidence for General Base Catalysis in an Ester Hydrolysis. I. Hydrolysis of an Alkyl Aminoacetylsalicylate[1]

BY EDWARD R. GARRETT

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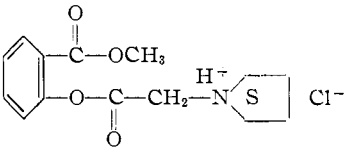
The protonated salicylate di-ester, methyl pyrrolidylacetylsalicylate hydrochloride is completely resistant to hydrogen ion catalyzed hydrolysis but is highly susceptible to general base catalysis as by water, hydroxyl and acetate ions. The effect of acetate ion on hydrolysis greatly exceeds ionic strength effects and is strong evidence of postulated general base catalysis of this ester. The product of hydrolysis in the *p*H range less than 7 is methyl salicylate.

The principal cause of the hydrolysis of the acyl esters of salicylic acid (*e.g.*, aspirin) in aqueous solution about *p*H 3 is hydrogen ion attack.[2-4]

The introduction of a protonated group such as ammonium ion in the salicylate di-ester should repel the proton necessary for acidic hydrolysis. This paper reports on the kinetics of hydrolysis of such a substance, methyl pyrrolidylacetylsalicylate hydrochloride (I).



I

Experimental

Potentiometric Titrations of Methyl Pyrrolidylacetylsalicylate Hydrochloride (I).—Titration of this material in water with 0.1000 *N* NaOH gave a pK_a' of 7.20 and an equivalent weight of 298 (theoretical 299.8). Titration in aqueous alcohol gave a pK_a' of 7.04 in 42% ethanol and an equivalent weight of 297.6 (theoretical 299.8). In both cases the sample did not precipitate during the titration.

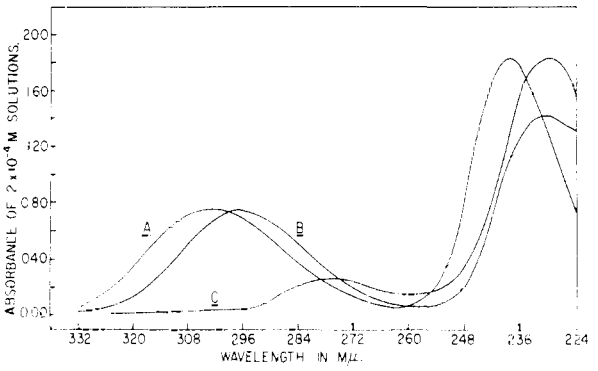


Fig. 1.—Spectra of methyl pyrrolidylacetylsalicylate hydrochloride and its hydrolysis products ($2 \times 10^{-4} M$).

Curve	Compounds and conditions
A	Methyl salicylate and salicylic acid at *p*H 1.0; completely hydrolyzed methyl pyrrolidylacetylsalicylate hydrochloride at *p*H 1.0 and 6.0 (from hydrolyses carried out on both *p*H 1.0 and 6.0)
B	Salicylic acid (sodium salt) at *p*H 6.0
C	Methyl pyrrolidylacetylsalicylate hydrochloride at *p*H 1.0 and 6.0

(1) Presented before the 131st National Meeting of The American Chemical Society, Miami, Florida, April 9, 1957.

(2) L. J. Edwards, *Trans. Faraday Soc.*, **46**, 723 (1950).

(3) L. J. Edwards, *ibid.*, **48**, 696 (1952).

(4) Edward R. Garrett, THIS JOURNAL, **79**, 3401 (1957).

Preliminary Rate Studies on the Aqueous Hydrolysis of Methyl Pyrrolidylacetylsalicylate Hydrochloride (I) **and Identification of Product.**—Sufficient I was weighed out to make up $10 \times 10^{-4} M$ solutions in an acid buffer (Buffer A) of *p*H *ca.* 1.0 (0.1304 *M* HCl and 0.0625 *M* KCl), solution A, and in a neutral buffer (Buffer B) of *p*H *ca.* 6.0 (0.1275 *M* acetic acid and 0.1225 *M* NaOH), solution B. These solutions were maintained in a constant temperature bath at 30.3°. Five ml. of solution A was diluted to 25 ml. with Buffer A and read ($2 \times 10^{-4} M$) at various intervals at 212–360 mμ on the Cary recording spectrophotometer, model 11, against Buffer A as the blank. Five ml. of solution A was added to 15 ml. of Buffer B and 5 ml. of 0.1 *N* NaOH to approximate the composition of Buffer B and this solution ($2 \times 10^{-4} M$) was run spectrophotometrically against Buffer B as the blank.

Five ml. of solution B was diluted to 25 ml. with Buffer B and run spectrophotometrically ($2 \times 10^{-4} M$) against Buffer B as the blank.

Five ml. of solution B was added to 15 ml. of Buffer A and 5 ml. of 0.23 *M* HCl to approximate the composition of Buffer A and this solution ($2 \times 10^{-4} M$) run spectrophotometrically against Buffer A as the blank.

The spectra of $2 \times 10^{-4} M$ methyl salicylate in water and $2 \times 10^{-4} M$ salicylic acid in Buffers A and B were also run.

No shift in spectra of I, either hydrolyzed at *p*H 1.0 or 6.0, was observed when aliquots of each solution were read at *p*H 1.0 or 6.0. This indicated that salicylic acid was not the end product.

When no change in spectra was observed with time, the spectra were coincident with those for salicylic acid at *p*H 1.0 (but not at *p*H 6.0) and methyl salicylate (Fig. 1). The conclusion is that methyl pyrrolidylacetylsalicylate hydrochloride hydrolyzes to methyl salicylate in the *p*H range 0–6. The apparent maximum for methyl salicylate was at 302 mμ.

Rates of Hydrolysis as Functions of *p*H and Buffer Concentrations.—Solutions of $10 \times 10^{-4} M$ methyl pyrrolidylacetylsalicylate hydrochloride (I) were made up in various aqueous buffers (Tables I and II) to determine the effect of *p*H and buffer concentrations on the hydrolysis of methyl salicylate. The appearance of methyl salicylate was determined by spectrophotometric readings on a Beckman DU at 302 mμ. A plot of the apparent dependence of rate on *p*H is given in Fig. 2. The effect of acetate concentration on pseudo first-order rates is shown graphically in Fig. 3.

Discussion

The pseudo first-order rate constants for the hydrolysis of methyl pyrrolidylacetylsalicylate hydrochloride to methyl salicylate in various buffers in the *p*H range 0–6 are given in Table I and plotted in Fig. 2 as an apparent function of *p*H. It is definitely shown that the protonated ester is not subject to specific hydrogen ion catalyzed hydrolysis since rate does not increase but actually decreases with decreasing *p*H and at lower *p*H values is apparently independent.

The increase of rate with *p*H cannot be fully accounted for by the probable mechanisms of hydroxyl ion attack on the protonated ester or hydrogen ion attack on the uncharged ester. These mechanisms would show a logarithmic increase of rate with *p*H. Figure 2 shows that after an initial in-

TABLE I

DATA ON THE HYDROLYTIC KINETICS OF METHYL PYRROLIDYLACETYLSALICYLATE HYDROCHLORIDE AS AN APPARENT FUNCTION OF *p*H IN VARIOUS BUFFER MEDIA

1	*p*H	0.90	3.01	4.04	5.03	6.25
2	k, sec.$^{-1}$	7.78×10^{-6}	8.00×10^{-6}	12.3×10^{-6}	20.2×10^{-6}	32.5×10^{-6}
3	Buffer	0.1304 *M* HCl	0.0570 *M* $HC_2H_3O_2$	0.1667 *M* $HC_2H_3O_2$	0.1163 *M* $HC_2H_3O_2$	0.1275 *M* $HC_2H_3O_2$
		0.0625 *M* KCl	(0.001 *M* $C_2H_3O_2^-$)	0.033 *M* NaOH	0.0837 *M* NaOH	0.1225 *M* NaOH
4	$^a\mu$	.....	0.001	0.033	0.0837	0.1225
5	$^b 2Az_1z_2\sqrt{\mu} \sim -\sqrt{\mu}$	.....	−0.032	−0.182	−0.290	−0.350
6	$^c k_{C_2H_3O_2^-}$	.....	2.01×10^{-4}	1.48×10^{-4}	1.23×10^{-4}	1.12×10^{-4}
7	^{d}Contribution of $\frac{k_{C_2H_3O_2^-}[C_2H_3O_2^-]}{(1 + K_a/[H^+]}$	.....	0.20×10^{-6}	4.88×10^{-6}	10.2×10^{-6}	12.3×10^{-6}
8	^{d,e}Contribution of $\frac{k_{H_2O}[H_2O]}{(1 + K_a'/[H^+]}$	7.78×10^{-6}	7.78×10^{-6}	7.78×10^{-6}	7.72×10^{-6}	7.00×10^{-6}
9	^{d,f}Contribution of $\frac{k_{OH^-}[OH^-]}{(1 + K_a/[H^+]}$	.....	.....	.....	1.1×10^{-6}	14×10^{-6}
10	Sum of assigned contribution of 7, 8 and 9, sec.$^{-1}$	7.78×10^{-6}	7.98×10^{-6}	12.7×10^{-6}	19.0×10^{-6}	33.3×10^{-6}

$^a \mu = {}^1/_2\Sigma c_i z_i^2$. $^b A \sim 0.5$, $z_1 = 1$, $z_2 = -1$. $^c \log k_{C_2H_3O_2^-} = -3.666 - \sqrt{\mu} + 0.53\mu$. $^d K_a = 6.31 \times 10^{-8}$, $pK_a = 7.20$. $^e k_{H_2O}[H_2O] = 7.78 \times 10^{-6}$. $^f \log k_{OH^-} \sim 3.3 - \sqrt{\mu}$.

TABLE II

DATA ON THE HYDROLYTIC KINETICS OF METHYL PYRROLIDYLACETYLSALICYLATE HYDROCHLORIDE AS A FUNCTION OF ACETATE BUFFER CONCENTRATION AT CONSTANT *p*H

1	$^a\mu = [Na^+] = [C_2H_3O_2^-]$	0.050	0.100	0.200
2	k, sec.$^{-1}$	14.64×10^{-6}	19.6×10^{-6}	27.5×10^{-6}
3	$^b k_{C_2H_3O_2^-}[C_2H_3O_2^-] = k - k_{H_2O}[H_2O]$	6.86×10^{-6}	11.8×10^{-6}	19.7×10^{-6}
4	$k_{C_2H_3O_2^-}$, l./mole/sec.	1.37×10^{-4}	1.18×10^{-4}	0.987×10^{-4}
5	$^c 2Az_1z_2\sqrt{\mu} \sim -\sqrt{\mu}$	−0.224	−0.316	−0.447
6	$^d[k_{C_2H_3O_2^-}]_0$, l./mole/sec.	2.160×10^{-4}	2.157×10^{-4}	2.163×10^{-4}
7	*p*H	4.71	4.65	4.65

$^a \mu = {}^1/_2\Sigma c_i z_i^2$. $^b k_{H_2O}[H_2O] = 7.78 \times 10^{-6}$. $^c A \sim 0.5$, $z_1 = 1$, $z_2 = -1$. $^d \log [k_{C_2H_3O_2^-}]_0 = \log k_{C_2H_3O_2^-} - 2Az_1z_2\sqrt{\mu} - f\mu = \log k_{C_2H_3O_2^-} + \sqrt{\mu} - 0.53\mu = 3.6655$.

dependence of rate on *p*H, the apparent dependence is most nearly linear.

However, inspection of the data of Table I clearly shows that the increase in rate over the low *p*H-independent rate nearly parallels the acetate ion concentration. The catalytic effect of acetate ion is clearly shown in the data of Table II and the rate plots of Fig. 3.

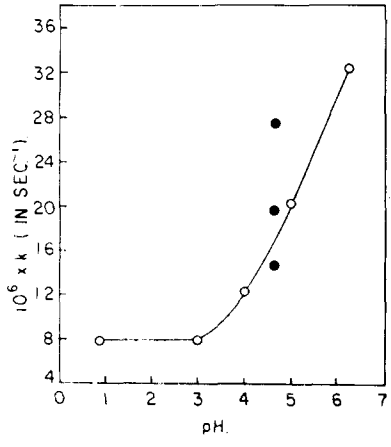


Fig. 2.—Apparent dependence of pseudo first-order rate constants (k in sec.$^{-1}$) of hydrolysis of methyl pyrrolidylacetylsalicylate hydrochloride as a function of *p*H. (The solid circles represent values obtained at constant *p*H with varying acetate concentration.)

The plausible mechanism is a general base catalysis of the protonated ester (R_3NH^+) and may be expressed as the pseudo first-order reaction

$$d[R_3N + R_3NH^+]/dt = -k[R_3N + R_3NH^+] = -(k_{H_2O}[H_2O] + k_{C_2H_3O_2^-}[C_2H_3O_2^-] + k_{OH^-}[OH^-])[R_3NH^+] \quad (1)$$

The specific rate constants for the general base-catalyzed hydrolysis of the protonated ester; specifi-

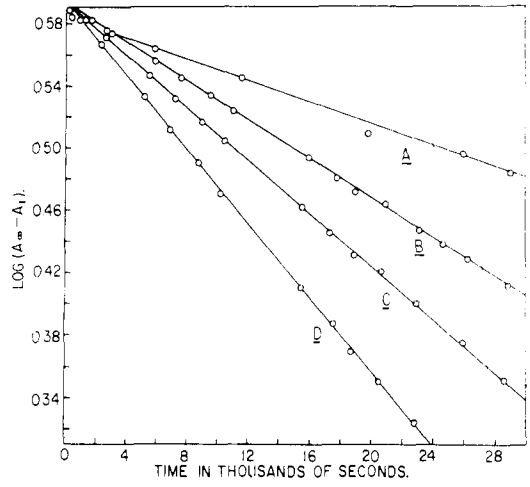


Fig. 3.—Effect of varying acetate ion on the rates of hydrolyses of $10 \times 10^{-4}M$ methyl pyrrolidylacetylsalicylate hydrochloride ($A_\infty = 3.900$)

Curve	$[HC_2H_3O_2]$	$[C_2H_3O_2^-]$	*p*H
A	0.0570	0.001	3.01
B	.050	.050	4.71
C	.100	.100	4.65
D	.200	.200	4.65

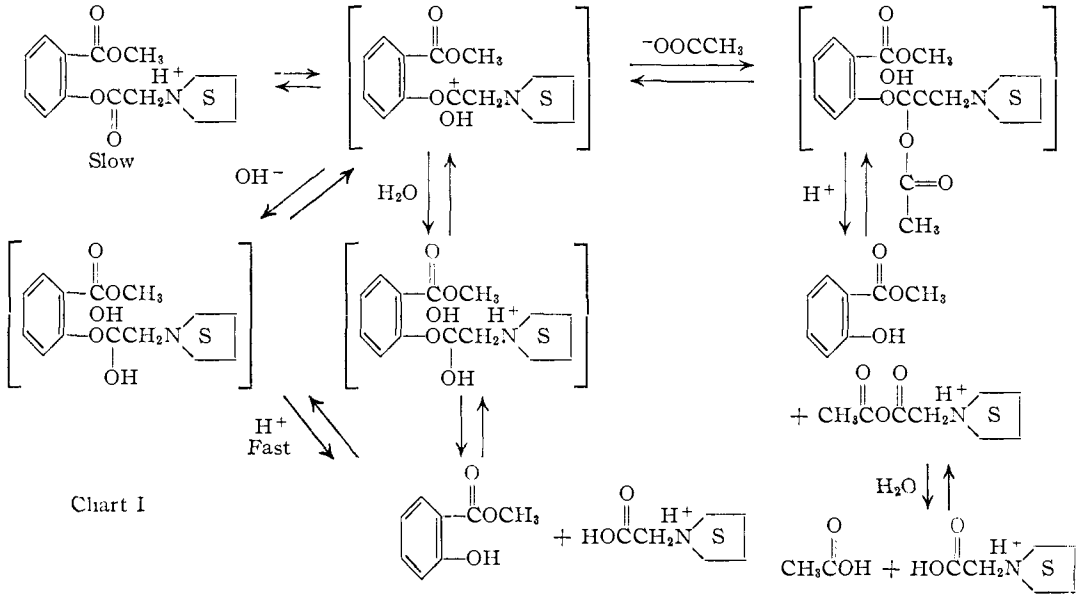


Chart I

cally by H_2O, $C_2H_3O_2^-$ and OH^-, may be derived by a method similar to Edwards[2,3] where

$$k = \frac{k_{H_2O}[H_2O] + k_{C_2H_3O_2^-}[C_2H_3O_2^-] + k_{OH^-}[OH^-]}{1 + K_a/[H^+]} \quad (2)$$

where

$$K_a = [R_3N][H^+]/[R_3NH^+] \quad (3)$$

The relative invariance of k in dilute hydrochloric acid and acetic acid, (Table I) indicates that chloride ion has low catalytic efficiency and that the rate in the former may be attributed to water attack on the protonated ester of 7.78×10^{-6} sec.$^{-1}$ Thus, the postulated resistance of the protonated ester to hydrogen ion catalyzed hydrolysis is substantiated.

The contribution of $k_{C_2H_3O_2^-}$ to the over-all rate may be derived from the rates obtained in the presence of various amounts of half-neutralized acetic acid (Table II and Fig. 3).

The ionic strength of the media is highly significant for a reaction between a negative (acetate ion) and a positive ion (protonated ester). This can be accounted for by the expression[5,6]

$$\log k_{C_2H_3O_2^-} = \log (k_{C_2H_3O_2^-})_0 + 2Az_1z_2\sqrt{\mu} + f\mu \quad (4)$$

where $(k_{C_2H_3O_2^-})$ is the rate constant at zero ionic strength in l./mole/sec., μ is the ionic strength, $z_1 = 1$, $z_2 = -1$, and $A \sim 0.5$ for the conditions of the hydrolyses. Consideration of the data in Table II permits evaluation of the constants so that

$$\log k_{C_2H_3O_2^-} = -3.6655 - \sqrt{\mu} + 0.53\mu \quad (5)$$

Thus, the contributions of $k_{H_2O}[H_2O]$ and $k_{C_2H_3O_2^-} \cdot [C_2H_3O_2^-]$ from equation 5 may be determined for the data of the hydrolyses in Table I and account for all of the rate for the pH range 0 to 4.5. On the assumption of hydroxyl ion attack on protonated ester contributing to hydrolysis above this latter pH, a first-order estimate of k_{OH^-} variation with ionic strength (Table I) would be

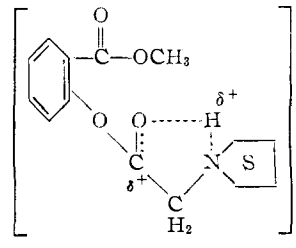
$$\log k_{OH^-} \sim 3.3 - \sqrt{\mu} \quad (6)$$

and k_{OH} is of the magnitude of 10^3 l./mole/sec. Thus, the over-all rate of hydrolysis of the protonated ester can be explained since the evaluated general base (acetate) catalytic constant of Table II permits estimates of over-all rates (item 10, Table I) that agree with the observed rates of an independent set of data (item 2, Table I).

It is, of course, possible that non-protonated ester may act as a catalyst for the protonated form and account for some of the hydrolysis attributed to the hydroxyl ion attack on the protonated ester.

The above-demonstrated general base catalysis of a presumed ester is unique, at least in magnitude and possibly even in fact, since considerable question has been raised as to whether general acid or base catalysis can be or has been observed in any ester hydrolysis.[7,8,9]

Of course, non-observance could be attributed to the relatively enormous reactivities of hydroxyl or hydrogen ion over any other Brönsted bases or acids for the esters studied.



A
(Ref. 11)

The simplest mechanism[10] to postulate is the ac-

(5) R. P. Bell, "Acid-Base Catalysis," Oxford University Press, London, 1941, Chapter I.

(6) E. S. Amis, "Kinetics of Chemical Change in Solution," The Macmillan Co., New York, N. Y., 1949, Chapter IV.

(7) R. P. Bell, ref. 5, page 80.

(8) K. J. Laidler, "Chemical Kinetics," McGraw-Hill Book Co., Inc., New York, N. Y., 1950, p. 298.

(9) After submission of this paper and after its presentation at the April, 1957, A.C.S. Meeting, it was found that adequate proof of general base catalyzed hydrolysis of esters already had been established simultaneously by T. C. Bruice and G. L. Schmir, THIS JOURNAL, **79**, 1663 (1957) (with a preliminary communication in *Arch. Biochem. Biophys.*, **63**, 484 (1956)) and by M. L. Bender and B. W. Turnquest, THIS JOURNAL, **79**, 1652, 1656 (1957). The latter authors had shown that it was not unique to one type of base.

(10) It is gratifying to see that this is directly analogous with the proposed mechanism of Bender and Turnquest (see ref. 9) and conforms to their postulated anhydride intermediate for general base

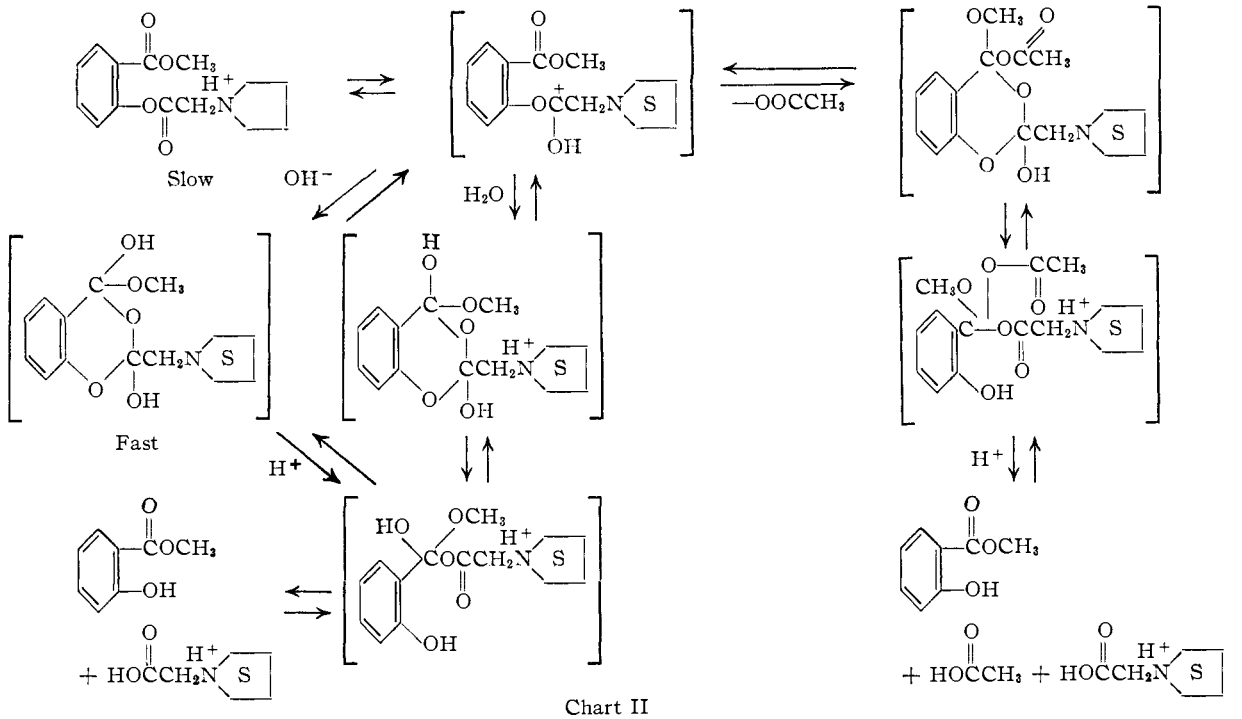


Chart II

tivation of the pyrrolidylacetyl carbonyl oxygen by the proton of the protonated nitrogen (either by adjacency[11] or direct transfer) (Chart I).

If the *o*-methyl carboxylate grouping is not involved in the mechanism, then general base catalysis may be expected in the hydrolysis of a dialkylaminoacetylphenate.

If the *o*-methyl carboxylate grouping is involved in the mechanism, an intramolecular condensation[4,12] may be postulated prior to hydrolysis. Of the two possible carbonyl activations, the most probable is in the pyrrolidylacetyl grouping as in II.

Acknowledgment.—Grateful appreciation is given to Dr. Fred Kagan and R. D. Birkenmeyer for the synthesis of the ester, to Miss Susan Theal for some of the titrations, and to Miss Kathryn Stimson for excellent technical assistance.

KALAMAZOO, MICH.

catalyzed hydrolysis by acetate ion. It also is consistent with their postulate that general base catalysis is of importance only for esters containing an alcohol that is a reasonably strong acid (pK_a <11), *i.e.*, methyl salicylate.

(11) The activation of the pyrrolidylacetyl carbonyl may also be by adjacency, *i.e.* as in preceding formula A.

(12) The possibility of a cyclic mechanism for salicylate ester hydrolysis cannot be ignored since this facilitates the explanation of the non-general base (*i.e.*, non-acetate) catalyzed "spontaneous hydrolysis" peculiar to acyl salicylates. (See Ref. (4) and references therein.)

[CONTRIBUTION FROM THE CLAYTON FOUNDATION FOR RESEARCH, THE BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS]

Synthesis of 2-Cyclohexene-1-glycine and 1-Cyclohexene-1-alanine, Inhibitory Amino Acid Analogs

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2-Cyclohexene-1-glycine and 1-cyclohexene-1-alanine were synthesized through the condensation of acetamidocyanoacetic ester and the appropriate halide followed by alkaline hydrolysis. In contrast tc the corresponding cyclohexane derivatives which were ineffective, 2-cyclohexene-1-glycine was found to be an antagonist of isoleucine for *Escherichia coli* 9723, and 1-cyclohexene-1-alanine was an antagonist of phenylalanine for *Leuconostoc dextranicum* 8086. The specificities of these amino acid antagonists have been correlated with the steric relationship of the cyclohexene group relative to the corresponding group of the natural amino acids.

Cyclopentaneglycine specifically inhibits the utilization of isoleucine[2] but 2-cyclopentene-1-glycine appears to be an antagonist of both isoleucine and valine in *Escherichia coli*.[3] The specificity of the cyclopentane analog as an isoleucine antagonist has been attributed to the slightly nonplanar structure of the cyclopentane ring. In *Leuconostoc dextranicum* 8086, cyclopentanealanine inhibits the utilization of leucine but not phenylalanine; this is in contrast to 1-cyclopentene-1-alanine which is an antagonist of phenylalanine but

(1) Rosalie B. Hite post-doctoral fellow, 1955–1956.

(2) W. M. Harding and W. Shive, *J. Biol. Chem.*, **206**, 401 (1954).

(3) R. L. Dennis, W. J. Plant, C. G. Skinner, G. L. Sutherland and W. Shive, THIS JOURNAL, **77**, 2362 (1955).