column (>50 plates); b.p. 130–132°. It was stored at 0° under nitrogen.

Azodiisobutyronitrile (Eastman Kodak Co.) was recrystallized several times from methanol; m.p. 102° dec.

Oxidation Procedure.—The initiator was added to the oxidation flask as an 0.1 *M* solution in benzene. The benzene was removed by evacuating at 15–20 mm. for approximately two hours. The cumene and chlorobenzene were added by pipet and the oxidation flask, which consisted of a heavy-walled erlenmeyer flask attached to a 30-cm. length of 16-mm. tubing, was connected to a gas manometer, evacuated and filled with oxygen several times. The flask then was attached to a reciprocating rack contained in a thermostated oil-bath and the rate of oxygen absorption measured by manual control of the mercury level in the gas buret.

Analytical Procedure. Hydroperoxides.—The hydroperoxide yields were determined by the stannous chloride procedure of Barnard and Hargrave[17] and carried out as follows: a 1–5-ml. aliquot of the oxidate was added to 10 ml. of 0.1 *N* stannous chloride solution containing 10 ml. of glacial acetic acid. The resulting mixture, contained in an erlenmeyer flask, was flushed with nitrogen, stoppered, and allowed to stand 1–2 hours in a nitrogen atmosphere with occasional shaking. Finally, the solutions were titrated with standard potassium triiodide solution. In each determination, a blank was carried through the entire procedure and the amount of hydroperoxide was thus determined by the difference in the titer of the blank and the solution containing the oxidate.

Acetophenone.—The yields of acetophenone were determined by infrared absorption utilizing a differential technique. In this procedure standards are prepared and their absorption at 5.9 μ measured in a 1-mm. cell utilizing a Perkin-Elmer model 21 spectrometer. Following this, these absorptions are balanced against a second set of standards placed in a matched cell in the reference beam of the spectrometer. In this manner, a calibration curve can be prepared from which it is possible to determine the amount of acetophenone present in the oxidates. The acetophenone was isolated and identified as its DNP derivative, m.p. 248–249°, mixed m.p. 249°; reported[18] 250°.

(17) D. Barnard and K. R. Hargrave, *Anal. Chim. Acta*, **5**, 476 (1951).

Dialkyl Peroxide.—This analysis was carried out essentially by the technique of Vaughan[7] and co-workers as modified by Hercules Powder Co., Inc.[8] Prior to analysis, an appropriately sized aliquot of the oxidate was heated in a nitrogen atmosphere to 110° for 3–5 minutes to decompose the residual AIBN. Then 50 ml. of glacial acetic acid, 3 g. of sodium iodide and 3 ml. of water were added and the whole heated in a nitrogen atmosphere at 110–115° for 1 hour. Finally, the resulting iodine was titrated in an atmosphere of carbon dioxide with standard thiosulfate solution. The difference between this determination and the stannous chloride determinations for hydroperoxide thus gave the amount of dialkyl peroxide present. The complete analytical procedure, including the pre-heating in the presence of AIBN, was checked with known quantities of pure di-α-cumyl peroxide and cumene hydroperoxide. It was found that the method was reproducible within $\pm 12\%$.

Vapor Phase Chromatographic Analyses.—Several of the oxidates were subjected to vapor phase chromatography utilizing an A-1 column at 120°. By this method, utilizing known solutions, both α-methylstyrene and α,α-dimethylbenzyl methyl ether were positively eliminated as oxidation products. Moreover, all of the peaks, with one exception, could be accounted for by comparison with the chromatograms obtained from known mixtures of acetophenone and cumene hydroperoxide. The one exception was a broad peak of very low intensity due to a compound with a relatively long retention time. Comparison with the chromatogram of a solution of α,α-dimethylbenzyl alcohol indicated that it could be due to this compound. Moreover, the intensity of this peak was slightly diminished upon addition of hexamethyldisilazane[6] as was the intensity of the corresponding peak from the chromatogram of the known solution containing the disilazane. The chromatograms of the known solutions containing enough α,α-dimethylbenzyl alcohol to correspond to an amount of oxygen absorbed in the oxidations of greater than 8% established this as an approximate upper limit for the amount of α,α-dimethylbenzyl alcohol which could be present in the oxidates.

(18) R. L. Shriner and R. C. Fuson, "Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948.

SCHENECTADY, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, YALE UNIVERSITY AND THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, THE JOHNS HOPKINS SCHOOL OF MEDICINE]

The Influence of Mechanism on the Apparent pK_a' of Participating Groups in Enzymic Reactions

BY THOMAS C. BRUICE[1] AND GASTON L. SCHMIR[2]

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It has become an accepted practice to employ the *p*H dependence curves of enzymic activity to determine the dissociation constants of groups involved in the catalytic processes and thereby establish their structural identity. However, kinetically determined acid dissociation constants may or may not reflect the nature of the participating groups and the values of the apparent dissociation constants will depend, among other things, on the intricacies of mechanism. Limiting our considerations to the mechanism of the bond making and breaking processes within the enzyme–substrate complex, the way in which *the constant of any equilibrium* (in addition to the dissociation constant) *occurring prior to the rate-determining step becomes a part of the kinetically determined pKa' value* is illustrated by considering six plausible mechanisms for an esteratic enzyme. The possible application of the concepts developed here to certain enzymic reactions is pointed out.

Introduction

To determine the nature of the ionizable groups which participate in enzymic reactions it is conventional to plot V_{max} at constant total enzyme concentration (or an equivalent rate expression) *vs.* *p*H and then to determine what pK_a' values fit best to the resultant *p*H dependency curve. Thus, in the reaction of trypsin with benzoyl-L-arginine ethyl ester the *p*H dependence curve has the shape of a dissociation curve for a single group of pK_{app} 6.25,[3] while for the reaction of α-chymotrypsin with acetyl-L-tryptophan ethyl ester and acetyl-L-tyrosine ethyl ester the kinetic data fitted well the theoretical curves for the dissociation of a single group of $pK_{app'}$ 6.7–6.74.[4] For the reaction of α-chymotrypsin with methyl hydrocinnamate the variation of rate with *p*H implicates groups of $pK_{app'}$ 7.2 and 8.0.[5] In the instance of the choline

(1) The Department of Physiological Chemistry, The Johns Hopkins School of Medicine.

(2) The National Institutes of Health, Bethesda, Md.

(3) H. Gutfreund, *Trans. Faraday Soc.*, **51**, 441 (1955).

(4) L. W. Cunningham and C. S. Brown, *J. Biol. Chem.*, **221**, 287 (1956).

(5) K. J. Laidler, *Disc. Faraday Soc.*, 93 (1955).

esterases the bell-shaped pH dependency curves have been rationalized in terms of the necessary participation of a group of pK_{app}' 6.9 to 6.7 in the free base form and one of 9.0 to 9.4 in the protonated form.[6] For the enzymes ficin[7] and papain[8] groups of pK_{app}' 4.5 and 8.5 best fit the pH dependency curves, etc.

Dissociable groups which may be presented by the enzyme[9] are the carboxyl (pK_a' 3.6–4.7), imidazole (pK_a' 6.4–7.0), amino (pK_a' 7.8–10.6), phenolic hydroxyl (pK_a' 9.6), sulfhydryl (pK_a' 9.4 to 10.8) and guanidino (pK_a' 11.9 to 12.0). The pK_a' of the various groups form a more or less continuous spectrum allowing rationalization of many simple or compound pH dependency curves in terms of the participation of specific groups. It is the purpose of this communication to indicate how the mechanism of the bond-breaking process within the Michaelis complex can influence the relationship of the actual dissociation constant (K_1) of a group involved in the catalysis to that determined kinetically (K_{app}) as well as the relation between the kinetic constant (k_1) of any rate-limiting step and the over-all experimentally determined rate constant (k_{app}). The general subject of the effect of pH on the gross Michaelis equilibria previously have been considered in detail.[10] For our present purpose we have employed hypothetical but feasible models of esteratic enzymes. It should be understood, however, that the conclusions reached here apply to enzymic reactions in general.

Considerable evidence has been accumulated in recent years to indicate that esters and amides are catalytically hydrolyzed by esteratic enzymes through a double displacement reaction involving an acylated enzyme intermediate.

$$E + RCOX \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{app}} E\text{-}CO\text{-}R + HX \quad (a) \quad (1)$$

$$E\text{-}CO\text{-}R \xrightarrow[H_2O]{k_h} E + RCOOH \quad (b)$$

It is possible that for any given enzyme k_1, k_2, k_{app} and k_h are pH dependent. Since we are interested in the processes occurring within the Michaelis complex leading to acyl enzyme (*i.e.*, equation 2) we may, for simplicity, assume k_{app} to be rate limiting

$$(ES_1 \longrightarrow ES_2 \longrightarrow ES_3 \dashrightarrow ES_n) \xrightarrow{k_{app}} E\text{-}CO\text{-}R + HX \quad (2)$$

and dependent on the dissociation of a single group (–BH) to its basic form (–B:) whereas the hydrolysis of acyl enzyme (k_h) as well as the dissociation of ES may be assumed to be non-pH dependent. With these simplifying assumptions the maximum velocity at high substrate concentration and any value of the hydrogen ion activity (a_H) would be

$$V_m = k_{app}E_T\left[\frac{K_{app}}{K_{app} + a_H}\right] \quad (3)$$

The observed first-order rate constant at any pH would then be

$$k_{obs} = \frac{V_m}{E_T} = k_{app}\left[\frac{K_{app}}{K_{app} + a_H}\right] \quad (4)$$

It follows from (4) that a plot of the observed pseudo-first order rate constant (k_{obs}) *vs.* pH will yield the sigmoid plot characteristic of the dissociation of a univalent weak acid. The point of inflection will be at $a_H = K_{app}$ and the maximum value of k_{obs}, reached asymptotically at high pH, will be k_{app}.

In Table I there are depicted six plausible mechanisms involving the participation of an ionizable (–BH) and a non-ionizable group (–OH) in the hydrolysis of the CO–X bond. Also included in Table I are the expressions relating the various K's and k's of the mechanisms to the experimentally determined constants. The conclusions reached concerning the values of K_{app} and k_{app} would not be altered if only one group were involved in the mechanisms and would only increase in complexity—conclusions remaining unchanged—if –OH were allowed to ionize. The use of two functional groups assists in the formulation of the mechanisms and is in accord with recent and long standing conjecture on the mechanisms of action of esteratic enzymes.[11]

It should first be noted that, regardless of the complexity of the mechanism, the simple first-order rate equation 3 is followed and that in each case a plot of k_{obs} *vs.* pH would yield a dissociation curve for a single group. Inspection of column four (Table I) reveals, however, that any equilibrium situation occurring prior to the rate-determining step(s) will be reflected in the relationship of K_{app} to K_1 and k_{app} to k_1 and, furthermore, that the value of K_{app} could vary considerably from the true dissociation constant of the ionizable group.

In case I, we have the most simple mechanism where –B: either displaces –X from –CO–X or acts as a general base catalyst of the Brönsted type to enhance attack of –OH on –COX. Since the only equilibrium involved is the dissociation of –BH, the kinetically determined dissociation constant equals that of –BH and the rate at low a_H is that of the limiting step (k_1). Many examples of the intramolecular catalysis of the hydrolysis of ester bonds by direct attack of –B: on –COX are known in which K_{app} is equal to K_1[12–15] and the catalysis of hydrolytic reactions *via* the Brönsted proton abstraction mechanism of I has been demonstrated.[16]

In case II, –B: or –OH do not replace –X in an SN2 type displacement but a stable tetrahedral compound is formed (ES_2H) whose collapse to acyl en-

(6) F. Bergmann, R. Segal, A. Shimoni and M. Wurzel, *Biochem. J.*, **63**, 684 (1956).

(7) S. A. Bernhard and H. Gutfreund, *ibid.*, **63**, 61 (1956).

(8) A. Stockell and E. L. Smith, *J. Biol. Chem.*, **227**, 1 (1957).

(9) J. T. Edsall and J. Wyman, "Biophysical Chemistry," Academic Press, Inc., New York, N. Y., 1958, p. 536; R. J. Cohn and J. T. Edsall, "Proteins Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, p. 85.

(10) See R. A. Alberty (*J. Cell. and Comp. Physiol.*, **47** (suppl. 1), 245 (1956)) for a summary of kinetic considerations of pH, pK_a' and the gross Michaelis constants.

(11) For the case of chymotrypsin see: (a) L. W. Cunningham, *Science*, **125**, 1145 (1957); (b) F. H. Westheimer, *Proc. Natl. Acad. Sci. (U. S.)*, **43**, 969 (1957); (c) B. J. Jandorf, *et al.*, *Disc. Faraday Soc.*, **20**, 134 (1955); (d) ref. 18 of this paper.

(12) G. L. Schmir and T. C. Bruice, THIS JOURNAL, **80**, 1173 (1958).

(13) M. L. Bender, F. Chloupek and M. C. Neveu, *ibid.*, **80**, 5384 (1958).

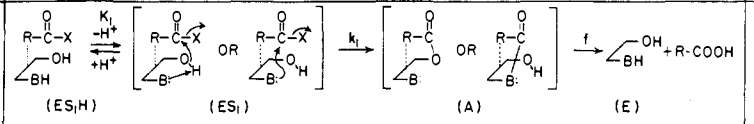
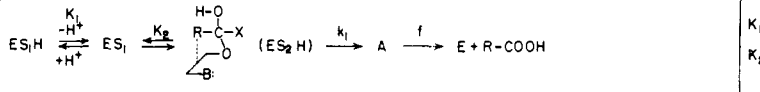
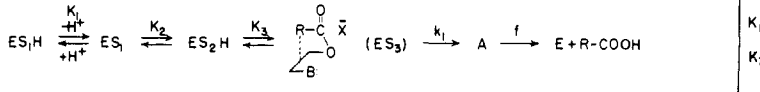
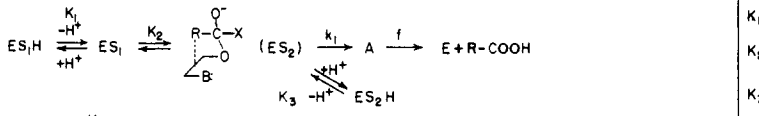
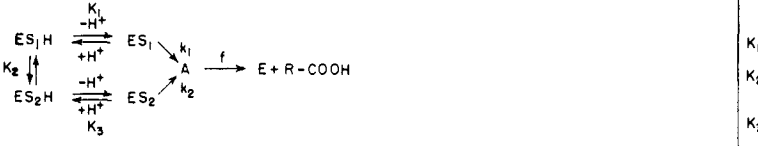
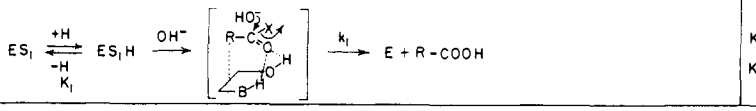
(14) L. J. Edwards, *Trans. Faraday Soc.*, **46**, 723 (1950); **48**, 697 (1952).

(15) H. Morawetz and P. E. Zimmering, *J. Phys. Chem.*, **58**, 753 (1954).

(16) W. Jenks, *J. Biol. Chem.*, **234**, 1280 (1958).

TABLE I
MECHANISMS FOLLOWING THE RATE EQUATION

$$V_m = k_{app} E_T \left[\frac{K_{app}}{K_{app} + a_H}\right]$$

	MECHANISM	EQUIL.	K_{app}	k_{app}
I		$K_1 = \frac{(ES_1)a_H}{(ES_1H)}$	K_1	k_1
II		K_1 $K_2 = \frac{(ES_2H)}{(ES_1)}$	$K_1(K_2+1)$	$\frac{k_1K_2}{K_2+1}$
III		K_1, K_2 $K_3 = \frac{(ES_3)}{(ES_2H)}$	$K_1(K_3K_2+K_2+1)$	$\frac{k_1K_3K_2}{K_3K_2+K_2+1}$
IV		K_1 $K_2 = \frac{(ES_2)}{(ES_1)}$ $K_3 = \frac{(ES_2)a_H}{(ES_2H)}$	$\frac{K_1K_3(K_2+1)}{K_1K_2+K_3}$	$\frac{k_1K_2}{K_2+1}$
V		K_1 $K_2 = \frac{(ES_2H)}{(ES_1H)}$ $K_3 = \frac{(ES_2)a_H}{(ES_2H)}$	$\frac{K_1+K_3K_2}{K_2+1}$	$\frac{k_1K_1+k_2K_2K_3}{K_1+K_3K_2}$
VI		K_1 $K_W = a_{OH} \cdot a_H$	K_1	$\frac{k_1K_w}{K_1}$

zyme becomes rate limiting. The rate of the acylation step would then be

$$V_m = k_1(ES_2H) \quad (5)$$

Since, $E_T = ES_1H + ES + ES_2H$, it follows from the equilibrium relationships of the various complex species that

$$E_T = ES_2H\left[1 + \frac{1}{K_2} + \frac{a_H}{K_2K_1}\right] \quad (6)$$

and

$$ES_2H = E_TK_2\left[\frac{K_1}{K_1(K_2+1) + a_H}\right] \quad (7)$$

Substituting this expression into (5) and multiplying denominator and numerator by the term $(K_2 + 1)$ leads to equations 8 and 9.

$$V_m = \frac{K_1K_2}{K_2+1}\left[\frac{K_1(K_2+1)}{K_1(K_2+1)+a_H}\right]E_T \quad (8)$$

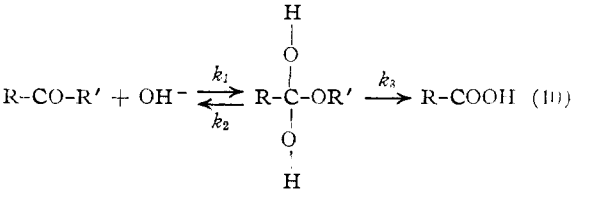
$$k_{obs} = \frac{V_m}{E_T} = \frac{K_1K_2}{K_2+1}\left[\frac{K_1(K_2+1)}{K_1(K_2+1)+a_H}\right] \quad (9)$$

Comparing (9) to (4), it is evident that $K_{app} = K_1(K_2 + 1)$ and $k_{app} = k_1k_2/(k_2 + 1)$.

It would be surprising if mechanism II were not operative in the case of certain esteratic enzymes. Thus, in the simple reaction of a nucleophile with an ester it can be shown (where nucleophile is hydroxide ion) that a tetrahedral intermediate does occur as a metastable compound[17] at a low steady state concentration.

In mechanism II, to have the concentration of the tetrahedral intermediate increase in relative concentration sufficiently to cause K_{app} to differ by one order of magnitude from K_1 requires K_2 to be greater

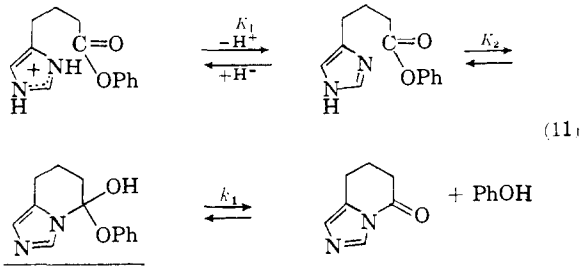
(17) M. L. Bender, THIS JOURNAL, **76**, 3350 (1954); **77**, 350 (1955).



(10)

where $k_{rate} = k_1k_3/(k_2 + k_3)$

than 10. This would not be unreasonable for an intramolecular reaction of the type which must occur within the Michaelis complex. An actual example of the operation of this mechanism in an intramolecular catalysis may be found in the hydrolysis of the phenyl esters of γ-(4-imidazolyl)butyric acid.[18] The hydrolysis of these esters occurs by an intramolecular nucleophilic attack of the non-protonated imidazolyl group[19] and in the case of the *p*-nitrophenyl ester the rate of hydrolysis is almost identical to that for the solvolysis of the ES complex of *p*-nitrophenyl acetate and α-chymotrypsin[20] which is also thought to occur with imidazole participation. For these phenyl esters the value of



(11)

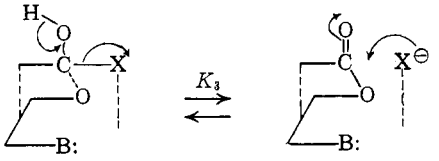
(18) T. C. Bruice and J. M. Sturtevant, *ibid.*, **81**, 2860 (1959).
(19) T. C. Bruice, *ibid.*, **81**, in press (1959).
(20) T. Spender and J. M. Sturtevant, *ibid.*, **81**, 1874 (1959).

pK_{app} becomes smaller as the electronat tracting nature of the substituent on the phenoxide radical becomes greater, *i.e.*

	pK_{app}	K_2
Phenol	6.91	0.55
p-Chlorophenol	6.69	1.53
m-Nitrophenol	6.79	1.06
p-Nitrophenol	6.24	6.22

However, by titrimetry, the pK_a' of the methyl ester is known to be 7.1. Since the replacement of methoxide by substituted phenoxide should have no effect on the acidity of the imidazolium group this is most reasonably explained as a kinetic effect of the type under consideration. The calculated values for K_2 are presented above.

Mechanism III differs from II in that the loss of –X from the tetrahedral complex is considered to be a reversible process. The reversibility of this step is made more comprehensible if it is assumed that binding of the substrate can occur at –X as well as on the acyl radical.



(12)

Since the acyl enzyme produced in the hydrolysis of an ester or amide is susceptible to nucleophilic attack, the process of external return of eliminated nucleophile is most probable. For the ester *n*-propyl γ-(4-imidazolyl)-thiolbutyrate a mass action effect on the elimination reaction by product has been demonstrated.[19] If ES_2H were required to lose a proton in an ionization step prior to collapse of the tetrahedral intermediate *via* ES_2, we would have case IV.

The mechanism of V differs from the simple case I in that an alternate route to A is envisioned. For the alternate route the –OH group is proposed to add across the carbonyl group of –COX without prior ionization of –BH, or what amounts to the same kinetically, the alkoxide ion attacks the protonated substrate. This alternate path is conceivable, being the most probable for N to O acyl transfer reactions[21,22] and can be seen as a true equilibrium situation in the intramolecular addition of the –SH group across the γ-L-glutamyl-L-cysteinyl peptide bond in glutathione[23,24] (in which case –SH replaces –OH in V). An example of the occurrence of compounds of type ES_2H as intermediates in the intramolecular catalysis of ester hydrolysis is found in the proposed mechanism for the hydrolysis of acetyl salicylate[25] and the catalysis of amide hydrolysis by protonated nucleophile (or kinetic equivalent, (*loc. cit.*) is observed in the intramolecular catalyzed hydrolysis of the amide of γ-(4-imidazolyl)-butyric acid,[18] phthalamide,[26] glycyl-L-asparagine and L-leucyl-L-asparagine[27] as well as for succinylsalicylic acid.[28] For K_{app} to vary by one order of magnitude from K_1 it would be necessary to have $K_1/K_3 = 10$ and for $K_2 = 1.0$.

Drawing attention to actual enzyme studies (equation 1), we see that both the acylation and deacylation steps could be dependent on the dissociation of –BH. If the two reactions were so dependent but differed in the intricacies of their internal equilibria, then the apparent dissociation constant for –BH would be different for each step. In practice it is often found that the apparent dissociation constants of BH are different for the two steps. Thus, Dixon and Neurath[29] report for δ-chymotrypsin a pK_{app} of acylation of 6.22 and a pK_{app} of deacylation of 6.96 when either *p*-nitrophenyl acetate or acetyl-L-tyrosine ethyl ester were employed as substrates. For α-chymotrypsin reacting with *p*-nitrophenyl acetate or dinitrophenyl acetate Gutfreund and Sturtevant[30] have reported pK_{app} of 6.7 and 7.3 for the acylation and deacylation steps, respectively. If the variations of K_{app} are due to additional equilibria prior to the rate-determining step then the lower pK_{app} for the acylation step would be in accord with a mechanism in which the ionization of –BH becomes fairly concerted with the formation of a tetrahedral intermediate while in the deacylation step the bond breaking process would have more SN2 character.[31] Some concern also has been expressed concerning the value of the apparent heats of ionization obtained kinetically for esteratic enzymes.[1a,4] On the basis that K_{app} is a composite constant it would be expected that ΔH_i would not only depend on K_1 but the additional equilibria which precede the rate-determining step(s).

Unlike mechanisms I to V, VI would not yield an acyl enzyme intermediate. However, since it has not been shown that an acyl enzyme is formed in the instance of all esteratic enzymes it is of some value to consider its consequence. The mechanism is one in which –BH and/or –OH is hydrogen bonded to the carbonyl group of –COX so that the activation energy for direct displacement of –X by hydroxide ion is lowered. Though, in this instance, the participating species is –BH, this fact cannot be determined kinetically and a plot of V_m *vs.* *p*H at constant E_T would yield the typical sigmoid curve commonly associated with the necessity of the basic form of –B: for enzymic activity. For this mechanism K_{app} would equal K_1 but k_{app} will be many orders of magnitude removed from the true rate constant k_1. Intramolecular catalyses of ester hydrolysis by this mechanism are known—an example is found in the hydrolysis of α-pyrrolidylacetyl salicylate.[32]

The mechanisms depicted in Table I do not, of course, include all possible modes of traveling from

(21) A. P. Phillips and R. Baltzly, THIS JOURNAL, **69**, 202 (1947).
(22) E. E. van Tamelen, *ibid.*, **73**, 5773 (1951).
(23) M. Calvin in "Glutathione," Academic Press, Inc., New York, N. Y., 1954, p. 3.
(24) D. Garfinkel, THIS JOURNAL, **80**, 4833 (1958).
(25) E. R. Garrett, *ibid.*, **79**, 3401 (1957).
(26) M. L. Bender, Y. L. Chow and F. Chloupek, *ibid.*, **80**, 5380 (1958).
(27) S. J. Leach and H. Lindley, *Trans. Faraday Soc.*, **49**, 921 (1953).
(28) H. Morawetz and I. Oreskes, THIS JOURNAL, **80**, 2591 (1958).
(29) G. H. Dixon and H. Neurath, *J. Biol. Chem.*, **225**, 1049 (1956).
(30) H. Gutfreund and J. M. Sturtevant, *Proc. Natl. Acad. Sci. (U. S.)*, **42**, 719 (1956).
(31) The pK change could also be due simply to H-bonding of OH and B before acylation but not after (J. M. Sturtevant).
(32) E. R. Garrett, THIS JOURNAL, **79**, 5206 (1957).

ES_1H to A but suffice to establish the point that pK_{app} can be greatly influenced by the bond making and breaking processes within the Michaelis complex (the intracacies of which are, to date, unknown for any enzyme).

Acknowledgments.—We wish to thank Dr. J. M. Sturtevant and Dr. H. Morawetz for interesting discussions concerning this work and the U. S. Public Health Service for their support.

BALTIMORE, MD.

[CONTRIBUTION FROM THE METCALF CHEMICAL LABORATORIES OF BROWN UNIVERSITY]

Norsteroids. II. Application of the Favorskii Rearrangement to the Preparation of A-Norpregnanes

BY NICHOLAS PAPPAS[1] AND HAROLD R. NACE

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Treatment of 2α-bromoallopregnane-3,20-dione with sodium methoxide in methanol gave, in 35–40% yield, a mixture of 2- and 3-carbomethoxy-A-norallopregnane-20-one. The 20-carbonyl group was converted to the ethylenethioketal group, and then these 2- and 3-carbomethoxy compounds were reduced to the 2- and the 3-methylol A-norallopregnane derivatives. The 2-isomer then was transformed to the 2-methylal compound, which on treatment with phenylmagnesium bromide gave the 2-phenylcarbinol derivative. After removal of the 20-ethylenethioketal blocking group, the carbinol was dehydrated and oxidized to give the known A-norallopregnane-2,20-dione.

In a previous publication[2] the preparation of A-norcholestane derivatives *via* the Favorskii rearrangement of 2α-bromocholestan-3-one was described. Similar results were obtained by Winternitz and de Paulet,[3] and Evans, de Paulet, Shoppee and Winternitz,[4] who also applied the reaction to the coprostane series.

Since the ring contraction step proceeded in high yield (72%), the reaction appeared to be attractive for the preparation of A-norpregnane derivatives. The results with 2α-bromoallopregnane-3,20-dione are reported here.

The most practical route to allopregnane-3,20-dione proved to be hydrogenation of pregnenolone in ethyl acetate with palladium-on-carbon, to give allopregnane-3β-ol-20-one in 87% yield. This compound then was oxidized with chromium trioxide in pyridine[5] to give an 88% yield of allopregnane-3,20-dione.

The dione next was brominated to 2α-bromoallopregnane-3,20-dione (I), as described by Rubin, Wishinsky and Bompard.[6] Although these authors made no assignment of conformation for the bromine, it is now possible to do so on the basis of the position of the infrared absorption band of the adjacent carbonyl group, as predicated by Corey.[7]

As predicted[7] for an A–B *trans*-3-ketone, bromination caused a shift of 19 $cm.^{-1}$ in the 3-keto absorption band (1712 → 1731 $cm.^{-1}$), and accordingly the 2-bromine must be equatorial and therefore α.

The Favorskii rearrangement was carried out by treating the 2α-bromoallopregnane-3,20-dione with sodium methoxide in methanol. By analogy with the same reaction in the cholestane series a mixture of A-noresters and non-rearranged by-products was expected. Accordingly the reaction mixture was saponified, thus making possible the separation of the sodium salts of the A-noracids from the non-acidic products. The nature of the products in the neutral fraction, which was mainly a mixture of hydroxy ketones, will be reported in a separate article.

For ease of handling, and for characterization, the A-noracid mixture was esterified with methanolic hydrogen chloride. The yield of A-noresters IIa and b, based on bromoketone, was 35–40% after purification by chromatography. The overall yield, based on allopregnane-3,20-dione, was increased from 22 to 34–36% if crude bromoketone was used directly in the Favorskii reaction without recrystallization. Repeated chromatography of the mixture, followed by fractional crystallization of the various fractions, did not yield a pure isomer, and the separation was therefore deferred to a later stage.

The most direct method of proving that ring contraction had occurred appeared to be the degradation of the A-noresters *via* the Barbier–Wieland method[8] to the known A-norallopregnane-2,20-dione[9a] (VIa) and A-norpregnane-3,20-dione[9b] (VIb).

Ethane dithiol was superior to ethylene glycol as a blocking group for the 20-ketone function and the mixed ethylenethioketals of the 2- and 3-carbomethoxy-A-norallopregnane-20-ones were obtained in 85% yield.

Treatment of this mixture with phenylmagnesium bromide gave the diphenylcarbinol derivatives IVa,b, which were treated with mercuric chloride, cadmium carbonate and acetone[10] to regenerate the 20-ketone function. Dehydration to the di-

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