0.05 and 0.025 *N* alcoholic hydrochloric acid or ammonia (prepared by passing the gases into alcohol), there was a marked drop in melting point of the recovered product. There was no change in the melting point of 1,2-dibenzoyl glycerol, however, when identical tests were made, giving further evidence of the greater stability of the β-type aromatic esters compared to the β-glycerides of fatty acids.

Solubilities.—In an earlier publication[19] it was noted that the solubility ratios of the α- and β-aromatic and aliphatic monoglycerides in ether were reversed, the aromatic α-esters being more soluble than the β-isomers, but in the aliphatic series, the α-esters were less soluble than the β-isomers. A similar reversal of solubility in alcohol will be noted from the data in Table I.

TABLE I

SOLUBILITIES

Compound	Solvent	Temp., °C. (±0.01°)	Soly. g. per 100 ml.
α-Monopalmitin	Alcohol	25	4.09
β-Monopalmitin	Alcohol	25	4.61
α-*p*-Bromobenzoate	Alcohol	26	16.05[a]
β-*p*-Bromobenzoate	Alcohol	26	4.41[a]
β-*p*-Bromobenzoyl-α,α′-benzylidene glycerol	Alcohol	26	0.50
	Ether	26	1.15
β-Palmityl-α,α′-benzylidene glycerol	Alcohol	26	1.75
	Ether	26	17.40

[a] Repeated from an earlier paper for comparison.[20]

Summary

A new method for the synthesis of α,β- or 1,2-diglycerides has been described, in which sodium glyceroxide and benzyl chloroformate served as intermediates. Good yields were obtained and the reaction conditions made it possible to avoid the common β- to α-shift in structure.

1,2-Dipalmitin, m. p. 64°, 1,2-dimyristin, m. p. 59° and 1,2-dibenzoate of glycerol, m. p. 59°, were prepared and their structures were verified by making derivatives of known constitution.

A solution of 1,2-dipalmitin in 0.1 to 0.025 *N* alcoholic hydrochloric acid and ammonia underwent a rapid change in structure, but the analogous 1,2-dibenzoate was stable under the same conditions of exposure.

PITTSBURGH, PENNA. RECEIVED AUGUST 4, 1939

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

On the Effect of Acids in the General Meaning of the Term on the Activity of Invertase

BY WALTER ABBOTT WISANSKY

That the value of the *p*H of a solution in which an enzyme is acting, is a most important factor in controlling the activity of the enzyme, is well known. For example, the enzyme invertase has its maximum power for inverting sucrose in a range of *p*H values surrounding *p*H 4.5. If the *p*H value is raised or lowered sufficiently away from *p*H 4.5, the activity of invertase diminishes.

The explanation of the *p*H effect on enzymatic activity has been sought for a long time. The ionization hypothesis of Michaelis[1] has been a much favored suggestion in spite of certain shortcomings.[2] On the basis of this hypothesis invertase is supposed to be active only in the un-ionized condition.

In considering the effect of *p*H changes, workers have only been concerned with the effect produced on enzymatic activity by changes in the thermodynamic activity of one acid, oxonium ion. Since Brönsted[3] and others have demonstrated the generality of the term "acid," it became of interest to determine whether or not any acid, in the general meaning of the term, could alter the activity of enzymes. It was thought that if neutral acids could exert some effect on the activity of the enzyme invertase under conditions which purported to keep the degree of ionization of the enzyme constant (conditions such as constant ionic strength, and constant *p*H value), then one might impute to oxonium ion a role similar to that played by neutral acids in controlling the enzymatic activity of invertase, beyond oxonium ion's assured role of controlling the degree of ionization of the protein enzyme.

Since in practically all enzyme work, buffers are employed to regulate the *p*H, and since buffer solutions contain acids in the general meaning of the term, here is a very convenient source of acids whose effects on the activity of invertase it is proposed to consider.

The effects of neutral buffer acids on the activity of invertase have been studied.

In brief, the plan pursued in studying the effects of buffer acids on the activity of invertase

(1) L. Michaelis and H. Davidsohn, *Biochem. Z.*, **35**, 386 (1911).
(2) J. M. Nelson, *Chem. Rev.*, **12**, 1 (1933).
(3) J. N. Brönsted, *ibid.*, **5**, 231 (1928).

was to determine the enzymatic activity in solutions of different buffer concentration, at various *p*H values, keeping the ionic strength of all the buffer solutions constant.

If the total buffer concentration of a solution is varied, the *p*H will remain practically the same and, if the ionic strength is kept constant, the degree of ionization of an enzyme (invertase in this case) in the solution should be unchanged.

Any observed loss in enzymatic activity obtained by increasing the buffer concentration in solution would then be due either to the increase in the concentration of neutral buffer acid or to the greater specific action of the potassium buffer salt as compared to potassium chloride. (The potassium chloride is added to the more dilute buffer solutions to build up the ionic strength to a given value.)

A salt like potassium acetate might well be expected to diminish the activity of invertase after the fashion of potassium chloride.[4] The effect of potassium acetate might be greater or less than that of potassium chloride. It was therefore necessary to determine whether or not any observed change in invertase activity with variations in total buffer concentration is due to buffer salt or buffer acid. One could distinguish between the two possibilities, as will be seen shortly, by studies of the influence of varying buffer concentrations on the activity of invertase at different *p*H values and at constant salt concentration.

As is evident from Fig. 1,[5] *p*H–activity curves for invertase obtained in the presence of different concentrations of acetate ion appear to spread fanlike from an origin near the *p*H optimum, as one passes to more acid *p*H values.

The results of other experiments not reported here indicate a similar effect when one substitutes a formate buffer in place of the acetate buffer Also this effect appears to be independent of the degree of purification of the invertase preparation used, being the same for preparations of very low time value[6] (*i. e.*, pure preparations of time values as low as 0.14 and 0.17 min.) as for a cruder commercial preparation known as Convertite.

In order to find out from Fig. 1 whether it is the difference in concentration of acetate ion or acetic acid which causes the difference between the two *p*H activity curves, consider the following.

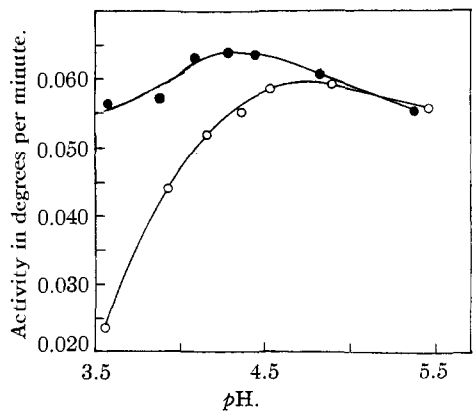

Fig. 1.—*p*H–activity curves based on data obtained in the presence of different concentrations of acetate ion; 10% sucrose; invertase preparation R(0.17); ionic strength, 0.2; ●, 0.02 molar total acetate; ○, 0.2 molar acetate ion.

Suppose the activity of invertase in a solution 0.02 molar in acetate ion is compared with that in a solution 0.05 molar in acetate ion, both solutions at *p*H 4.6. The mole fraction of acetate ion is 0.5. Therefore there is in each solution, respectively, 0.02 and 0.05 molar acetic acid. The activity of invertase is next examined at a more acid *p*H where the mole fraction of acetate ion is now 0.1. There is, in the dilute acetate solution 0.02 molar acetate ion and 0.18 molar acetic acid, and in the more concentrated buffer solution there is 0.05 molar acetate ion and 0.45 molar acetic acid. The difference in concentration of neutral acid has gone up from 0.03 molar at *p*H 4.6 to 0.27 molar at the more acid *p*H.

In Fig. 1, just as in the above example, parallel to the increase in the activity difference between the two *p*H–activity curves, there is a simultaneous increase in the difference of the acetic acid concentration as the *p*H is lowered. This points to the neutral acid as the cause of the loss in activity of invertase, as the buffer concentration is increased.

As further evidence that it is the neutral acid which causes the activity loss rather than the greater inhibitory effect of acetate ion over chloride ion, at *p*H values acid to 4.5, it is seen that on the alkaline side of the *p*H optimum practically no difference in the activity as measured in solutions of different buffer concentrations

(4) M. Washburn, Dissertation, Columbia University (1932).

(5) The numerical data are not given for brevity's sake. An idea of the accuracy of the measurements may be had from Table I in the experimental section. The activities upon which the figures in this paper are based were usually about the order of magnitude of 0.0500 degree per minute, it having been found that such a magnitude is conducive to greatest accuracy.

(6) C. O'Sullivan and F. W. Thompson, *J. Chem. Soc.*, **57**, 834 (1890).

is present. If acetate ion were a more effective retardant of invertase activity than chloride ion, then on the alkaline side of the *p*H optimum this effect should again be in evidence since the enzyme is quite sensitive to salt on the alkaline side as on the acid side of the *p*H optimum. There is, however, little difference between the "potassium acetate" curve (concentrated buffer), and "potassium chloride" curve (dilute buffer) in this region of *p*H.

As a final check, *p*H–activity curves were obtained in the presence of constant concentrations of acetic acid. From Fig. 2 it is evident that, to a first approximation, a given increase in acetic acid concentration causes a certain loss in activity independent of *p*H when this is in the optimum zone.

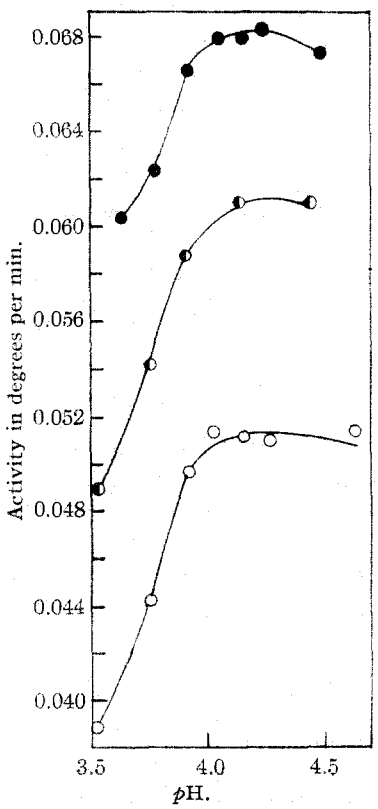

Fig. 2.—*p*H–activity curves based on data obtained in the presence of constant concentrations of acetic acid; 5% sucrose; invertase preparation, commercial Convertite; ionic strength 0.5; ●, 0.01 molar acetic acid; ◐, 0.2 molar acetic acid; ○, 0.5 molar acetic acid.

It is again plain that neutral acetic acid can decrease the activity of invertase.

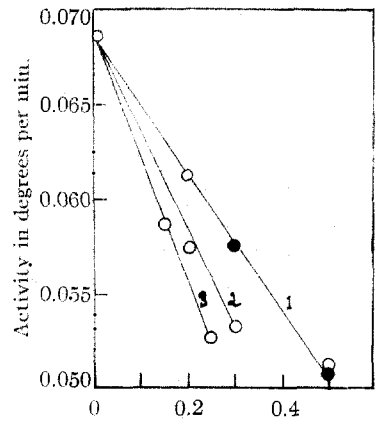

Fig. 3.—Data showing the variation of invertase activity with concentration of acid; 5% sucrose; open points refer to Convertite preparation; closed points refer to preparation R(0.14); curve 1, acetic acid, ionic strength 0.5; curve 2, formic acid, ionic strength 1.7; curve 3, acid phthalate ion, ionic strength 0.5.

In Fig. 3 data are plotted showing the effects of formic acid and acid phthalate ion (a charge type acid) on the activity of invertase as compared to the data on the retardation produced by acetic acid. The activities at zero buffer concentration have been equated. The *p*H values all lie in the optimum zone. The formic acid buffer experiments were performed at an ionic strength of 1.7 since at *p*H values close to the optimum *p*H 4.5 the buffer is practically neutralized and since a fairly large formic acid concentration was required. Because of the larger salt concentration present, a comparison of the effect of formic acid on the activity of invertase with that of acetic acid and acid phthalate ion may not be justified. The effect of these two latter acids on the activity of invertase was studied in 0.5 molar salt solutions.

There is a linear relation between the concentration of acid and the drop in invertase activity. This may be more apparent than real, however, since no correction was applied to correct for the loss in activity that accompanied the displacement of water by acids and salts.[7]

While acetic acid is a stronger acid than acid phthalate ion, as evidenced by their dissociation constants, the latter is the more effective retardant.

(7) It was shown by Nelson and Schubert[8] that decreasing the water concentration even in an essentially aqueous solution produces a loss in the activity of invertase.

(8) J. M. Nelson and M. Schubert, THIS JOURNAL, **50**, 2188 (1928).

Discussion

It is evident from the above that acids in the general meaning of the term can diminish the activity of invertase. It is probable that the effect can be extended to other enzymes. It is therefore suggestive to postulate something as to the meaning of the acid effect.

Mirsky and Pauling[9] hypothesize that proteins are held in their particular configurations through the medium of hydrogen bonds. In the light of this idea, it is suggested that in the case of the retardation of invertase activity by acids, these latter form intermolecular hydrogen bonds with certain electronegative groups in the enzyme protein, which groups are bound by intramolecular hydrogen bonds to other similar groups in the protein. There is a sort of competition in this respect. The result is a breaking of some of the weaker bonds in the protein with the further result that the enzyme passes into some more unspecified form of less enzymatic activity.

Since oxonium ion is an acid, it too might be expected to exert its effect upon the enzyme in a manner similar to that of neutral acids. The first step in the attack of oxonium ion would be the formation of an intermolecular hydrogen bond with the concurrent breaking of an intramolecular hydrogen in the protein enzyme. This would again result in the formation of a more unspecified configuration possessing less activity as an enzyme. The fact that water might subsequently be dissociated from the newly formed hydrogen bond complex, yielding a protein with an extra proton (*i. e.*, a change in the degree of dissociation of the enzyme) may be of secondary importance. In short it is suggested that changes in oxonium ion concentration in altering the activity of invertase do so by bringing about changes in the enzyme protein of a deeper sort than mere ionization changes.

Experimental

(1) The salts and acids used in this investigation were C. P. grade. They were used without further purification. The sucrose used was obtained in a grocery store.

(2) In the experiments on buffer acids it was necessary to know accurately the amount of buffer acid, the concentration of salt (buffer salt and potassium chloride) and that of sucrose in the reaction mixture.

Buffer solutions of known acid and salt concentrations were prepared by standardizing the acid constituent against potassium hydroxide. The alkali was standardized against potassium acid phthalate; 4 molar solutions of the acids were kept in resistance glass bottles; 1 and 2 molar solutions of potassium hydroxide were kept in paraffin lined bottles protected from carbon dioxide. In preparing the buffer a known volume of buffer acid of accurately known concentration was titrated with standard alkali until a given fraction was neutralized. From a knowledge of the total volume of solution, the buffer salt and acid concentration was calculated. A definite volume of the solution so prepared was pipetted into a 250-cc. volumetric flask, keeping in mind the concentration of buffer required to exist in the final reaction mixture. To the volumetric flask was added sucrose and potassium chloride (in sufficient amount to give the required ionic strength in the reaction mixture); 25.00 ± 0.05 cc. of invertase solution was pipetted in six seconds into 200 cc. of solution prepared as above. At recorded time intervals 25.00 cc. of reaction mixture was pipetted into bottles containing sufficient alkali to stop the reaction (*p*H alkaline to phenolphthalein). After allowing five to fifteen minutes for mutarotation, the rotations of the samples were determined. The activity was expressed as the average change in rotation in degrees per min. Usually four constants were used in calculating the average activity.

In calculating the ionic strength, the contribution due to the ionization of the acid constituent of the buffer was neglected, its contribution being too small to affect the activity of invertase when the ionic strength is high (see Table I).

TABLE I

Ionic strength	Enzymatic activity and a. d.	*p*H
0.20	0.380 ± 0.001	3.94
.22	.375 ± .001	3.96
.23	.375 ± .003	3.96

(3) *p*H measurements were made using both the glass and hydrogen electrodes.

(4) **Invertase Preparations.**—The author is grateful to Mr. Stanley Lewis of these laboratories for the preparation of the following highly purified yeast invertase preparations, RaDKDADSDAD[10] time value 0.14 min. known in this paper as R(.14), RaDAceSKSAD time value 0.17 min. known in paper as R(.17). A commercial invertase preparation known as Convertite also was used.

(5) Hydrolyses were conducted at 25.00 ± 0.02°.

Acknowledgment.—The author wishes to express his appreciation to Professor J. M. Nelson for his friendly advice and constructive criticism throughout the course of this investigation.

Summary

1. It has been demonstrated that acids in the general meaning of the term can retard the enzymatic activity of invertase under conditions which purport to hold the degree of ionization of the enzyme constant (*i. e.*, conditions such as constant ionic strength and constant *p*H).

2. It has been suggested that oxonium ion and

(9) A. E. Mirsky and L. Pauling, *Proc. Nat. Acad. Sci. U. S.*, **22**, 439 (1936).

(10) For the meaning of the letters which refer to steps in the purification see Lutz and Nelson, *J. Biol. Chem.*, **107**, 169 (1934).

acids in general may alter the activity of an enzyme by causing the rupture of intramolecular hydrogen bonds in the enzyme protein. The results of such bond breaking may be the arising of modified forms of the enzyme with more internal degrees of freedom, possessing less catalytic activity.

NEW YORK, N. Y. RECEIVED SEPTEMBER 27, 1939

[CONTRIBUTION FROM THE BASIC SCIENCE RESEARCH LABORATORY OF THE UNIVERSITY OF CINCINNATI]

Kinetics of Polyesterification: A Study of the Effects of Molecular Weight and Viscosity on Reaction Rate[1]

BY PAUL J. FLORY

Introduction

Investigators in the field of macromolecular chemistry have been prone to attribute the apparent sluggishness of some polymer reactions either to an inherent diminished reactivity of large molecules, or to the high viscosity of the reaction medium. Various arguments have been advanced in an effort to show theoretically that increase in size of the molecule and increase in viscosity should decrease reactivity.[2,3] However, no direct experimental evidence has been advanced to prove even the existence of an effect of either molecular weight or viscosity on reactivity of polymer molecules.

The problem of the effect of viscosity on reaction rate of liquid phase reactions in general has been the subject of frequent discussion,[4] but again conclusive experimental evidence does not seem to be available. Moelwyn-Hughes[5] has concluded that the rate of collision between solute and solvent molecules should increase proportionally with viscosity and, hence, the rate of a reaction between solute and solvent should be proportional to viscosity also. More recently Rabinowitch and Wood[6] have shown with exceptional clarity that one should expect no effect of viscosity on reaction rate, except at very high viscosities (very low rates of diffusion) or when a large fraction of collisions leads to reaction.

The study of the kinetics of a condensation polymerization offers a splendid opportunity to observe the effects of molecular weight and viscosity on reaction rate. Both molecular weight and viscosity increase continuously as such reactions proceed. It should be possible, therefore, to observe the effects of molecular weight and viscosity on reaction rate, if such effects exist.

The present investigation has been primarily concerned with the kinetics of polyesterification reactions between glycols and dibasic acids. The kinetics of several esterifications involving monofunctional reactants have been investigated also, in order that the polyesterification reactions could be compared with similar reactions in which neither molecular weight nor viscosity underwent appreciable change. All of these reactions have been followed by titration of the total free carboxyl in samples removed from the reaction mixture at suitable intervals.

In contrast to monofunctional esterification where there are only two species of reactants, many species of molecules are present during polyesterification. Any one of these species bearing an hydroxyl group may react with any other bearing a carboxyl; the product of this reaction may react with another molecule, etc. Many reactions are occurring simultaneously: *i. e.*, monomers react with monomers, dimers, trimers, etc., dimers react with monomers, dimers, trimers, etc., trimers react with monomers, etc., etc. A complete kinetic analysis, treating each of these reactions individually, is obviously hopeless. All of these reactions, however, are chemically identical, and their rates may differ only in so far as reactivity is affected by molecular weight. Instead of attempting to unravel the maze of individual reactions, one may consider merely the reaction of all functional groups, disregarding the size of the molecule to which each group is attached. If, as usually has been supposed, reac-

(1) A portion of this work was included in a paper presented before the Organic Plastics Section, Paint and Varnish Division of the American Chemical Society at the Boston Meeting, September 14, 1939.

(2) C. E. H. Bawn, *Trans. Faraday Soc.*, **32**, 178 (1936).

(3) H. Dostal, *Monatsh.*, **67**, 63 (1935); **70**, 409 (1937); H. Mark, *Nature*, **140**, 8 (1937).

(4) See E. A. Moelwyn-Hughes, "Kinetics of Reactions in Solution," Oxford Univ. Press., New York, N. Y., 1933, p. 51.

(5) Reference 4, pp. 19, 159 *et. seq.*

(6) E. Rabinowitch and W. C. Wood, *Trans. Faraday Soc.*, **32**, 1381 (1936).