14. 6-Methyl-8-(2,2,6-trimethylcyclohexyl)-octanone-2 (XI).—This saturated ketone was prepared from the mono-unsaturated ketone XVII, and from the triunsaturated ketone X, by catalytic hydrogenation.

(a) Twenty-seven grams of mono-unsaturated ketone and 0.10 g. of Adams catalyst in glacial acetic acid solution absorbed the calculated amount of hydrogen in sixty-five minutes. After distillation the physical properties were: $n^{24}D$ 1.4690, d^{24}_4 0.8896, M_D found 83.34, calcd. 83.14.

The semicarbazone after recrystallization from methyl alcohol melted at 113.5°. Karrer gives 113.5–114° for this compound.

Anal. Calcd. for $C_{19}H_{37}N_3O$: C, 70.6; H, 11.53; N, 13.00. Found: C, 70.65, 70.75; H, 11.33, 11.30; N, 12.76, 12.63.

(b) 2.38 g. of tri-unsaturated ketone X, and 0.05 g. of Adams catalyst in 30 cc. of glacial acetic acid absorbed 605 cc. of hydrogen in about ten hours. The material was fractionated into two fractions, both of which gave good yields of a semicarbazone melting at 113.5–114°; mixed m. p. with that above was 113.5–114°.

Summary

This paper describes the preparation in five steps of 6-methyl-8-(2,2,6-trimethylcyclohexenyl-Δ^6)-octadien-5,7-one-2 from β-ionone, and the corresponding 6-methyl-8-(2,2,6-trimethylcyclohexyl)-octanone-2 from tetrahydroionone. The initial step in the synthesis, addition of acetylene to the ketones, was accomplished using potassium *t*-amylate as condensing agent. This is a new reaction in the case of β-ionone, an α,β-unsaturated ketone; and in the case of tetrahydroionone resulted in a faster reaction and improved yields for the acetylene condensation. The 6-methyl-8-(2,2,6-trimethylcyclohexenyl-Δ^6)-octadien-5,7-one-2 is of special interest as an intermediate in our projected synthesis of dihydrovitamin A.

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[CONTRIBUTION FROM THE DIVISION OF CHEMISTRY, NEW YORK STATE AGRICULTURAL EXPERIMENT (GENEVA) STATION]

Water Relations of Enzymes. I. Influence of Viscosity on Invertase Action[1]

BY Z. I. KERTESZ

Because the importance of water in biological reactions has been much emphasized recently, an investigation of water relations of enzymes was undertaken; and since much information was available on invertase and its kinetics, this enzyme was selected for the work. The present paper deals with the influence of viscosity on the rate of sucrose inversion by invertase.

Achalme and Bresson[2] and later Colin and Chaudun,[3] claimed that the viscosity is a governing factor in enzyme reactions and that the rate of sucrose inversion by invertase is proportional to the fluidity of the medium. Schubert[4] and Nelson and Schubert[5] showed the faultiness of this assumption. According to the latter authors the concentration of water is a factor determining the rate of inversion. Auden and Dawson[6] were also unable to confirm the findings of the French authors.

In all the experiments of the above workers high concentrations of sucrose, glycerol and alcohol were used to alter the viscosity of the medium. This method is not entirely satisfactory in a study of the influence of viscosity because of the great changes that are produced in the concentration of the water and possible inhibiting action of glycerol and alcohol. Colin and Chaudun[7] determined the rate of inversion in the presence of gelatin and gelose and again found the previously claimed relation between fluidity and velocity of the reaction. Several serious objections might be raised to their work, however. First, the determination of changes of optical rotation in sucrose–gelatin mixtures is uncertain and has been found unworkable by the author. Second, a partial solidification of the gelatin sols might introduce serious changes in the velocity of the reaction, as shown by Freiberger[8] for amylolysis. Third, gelatin itself has been found by Filipowicz[9] to influence enzyme action (amylolysis) greatly. Finally, the whole range of viscosity change was very small, that of the medium being only doubled by the added materials.

(1) Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Article No. 46. Presented at the "Symposium on the Chemistry of Enzymes" at the Cleveland meeting of the American Chemical Society, Sept. 12, 1934.

(2) Achalme and Bresson, *Compt. rend.*, **152**, 1328, 1420, 1621 (1911).

(3) Colin and Chaudun, *Bull. soc. chim. biol.*, **4**, 272 (1922); *J. chim. phys.*, **20**, 4719 (1923).

(4) Schubert, Diss., Columbia Univ., 1928.

(5) Nelson and Schubert, THIS JOURNAL, **50**, 2188 (1928).

(6) Auden and Dawson, *Biochem. J.*, **25**, 1909 (1931).

(7) Colin and Chaudun, *Bull. soc. chim. biol.*, **11**, 258 (1929).

(8) Freiberger, *Biochem. J.*, **25**, 705 (1931).

(9) Filipowicz, *ibid.*, **25**, 1874 (1931).

It was desirable, therefore, to determine the rate of sucrose inversion in the presence of a non-toxic colloidal material which produces high apparent viscosity in low concentrations without causing appreciable change in the concentration of water and without the danger of setting to gel. Purified citrus pectin was used for this purpose. Apparent viscosities in the reaction mixture as high as twenty-five times that of water have been attained. Table I presents the results of a typical set of determinations.

TABLE I

INFLUENCE OF VISCOSITY ON THE VELOCITY OF SUCROSE INVERSION BY INVERTASE AT 20°

Mixture no.	1	2	3	4	5
Rel. visc. (water:1)	1.14	2.95	6.13	11.32	18.50
Pectin, %	0.00	0.40	0.79	1.28	1.71
Minutes	Rot.°	Rot.°	Rot.°	Rot.°	Rot.°
15	+2.28	+2.23	+2.25	+2.29	+2.25
30	2.18	2.10	2.08	2.01	2.13
60	1.74	1.78	1.72	1.74	1.74
120	1.08	1.11	1.19	1.13	1.12
180	+0.63	+0.58	+0.58	+0.58	+0.57
300	+ .01	+ .08	− .01	+ .01	+ .03
450	− .16	− .09	− .17	− .17	− .19

Initial Rot. = 2.517°. Final Rot. (calcd.) = −0.735°.

If the assumption of Achalme and Bresson and of Colin and Chaudun is correct, the decrease of

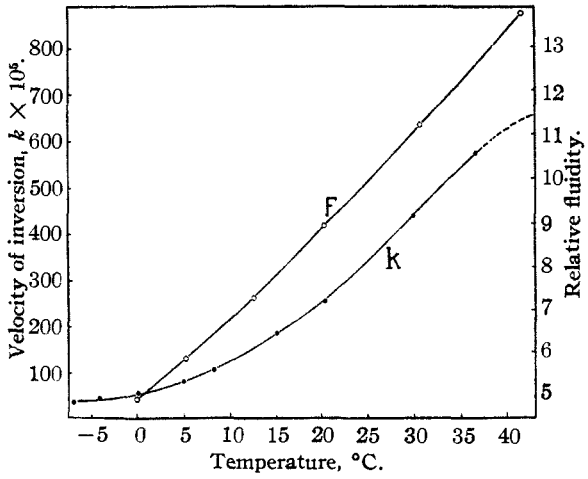

Fig. 1.—Rate of inversion (k) and relative fluidity (F) of sucrose–invertase mixtures.

the optical rotation in mixture 1 should have been much more rapid as in mixture 5 which had an apparent relative viscosity of 18.5. The results, however, showed no significant difference in the rate of reaction in the five mixtures. This observation is in direct contradiction to the theory of the French authors and to the experimental results of Colin and Chaudun.[7] A similar behavior of other enzymes can be presumed since in working with enzymes acting upon highly viscous substrates such as gelatin or pectin, no increase in the rate of reaction or constants could be observed after partial decomposition of the substrate and diminution of the viscosity.

It seems to be established that there is no correlation between velocity of invertase reactions and viscosity so far as viscosity caused by introduced colloid is concerned. It remains to be seen, however, how closely changes caused in the viscosity by temperature alterations can be correlated to the rate of invertase action. In Fig. 1 the results of a set of determinations are shown in which the velocity of the inversion and fluidity have been determined at temperatures from −6.8 to +36.8°.

It will be noted that the relative fluidity appears to be approximately a linear function of the temperature. The temperature coefficients of enzyme reactions rapidly approach 1 at temperatures where heat inactivation proceeds. There is a decrease of this constant for invertase at temperatures near and below 0°, as shown previously by the author.[10] Incidental correlation between velocity and fluidity might be found for a fraction of the range from 20 to 35° where the relation between velocity and temperature is a linear function because the temperature coefficient is fairly constant. If the theory of the French authors holds true, the correlations should be best expressed at 0 to 10° where there is no heat inactivation of the enzyme. However, this is not the case. Consequently, neither variations in the apparent fluidity brought about by the addition of materials nor changes caused by the alteration of temperature are directly proportional to the velocity of inversion in the same mixture.

These findings have also some biological significance. Belehradek[11] has put forward the theory that the rate and temperature coefficients of biological reactions depend solely on the rate of diffusion which is strictly related to the viscosity of the medium. Accordingly, the temperature coefficients of biological reactions would be just the coefficients of corresponding viscosities. Stiles[12] contradicted this hypothesis and showed that it is incorrect in the case of chemical reactions. From the present work it seems to be established that Belehradek's theory is invalid in

(10) Kertesz, *Z. physiol. Chem.*, **216**, 229 (1933).
(11) Belehradek, *Protoplasma*, **1**, 243 (1929).
(12) Stiles, *Biol. Review*, **5**, 171 (1930).

the case of enzyme reactions, specifically in the case of invertase where the temperature coefficients of velocities cannot be correlated to the temperature coefficients of fluidities. It has been found by other workers[13] that no essential difference exists between invertase action *in vitro* and *in vivo* or whether in solution or absorbed.[14] Thus viscosity cannot be the determining factor in the rate of invertase action in biological enzyme reactions. It is plausible to presume a similar behavior of other enzymes.

Experimental Part

The reaction mixture used in all experiments contained 4.72% sucrose, 0.2% invertase solution ("Difco" standardized), and 20% 0.2 *N* acetate buffer (*p*H 4.5). Great care was exercised to adjust the *p*H of the pectin solutions to 4.5 before mixing with the other components of the reaction mixtures. The monomolecular constants were calculated from polarimeter readings made on samples from the reaction mixtures clarified by lead acetate and alcohol. The values used for "*k*" in Fig. 1 are the averages of at least six individual constants, but any other way of expressing the velocity would give a figure of similar shape. The apparent viscosity was determined by the use of the Ostwald viscosimeter, the value for water at 20° being 1'9.5".

After this paper was submitted for publication, a paper by M. Niculescu appeared [*Bull. soc. chim. biol.*, **16**, 903 (1934)] giving evidence that changes produced in the apparent viscosity of the medium by the use of gelatin or salep is without effect on the fermentation of glucose by yeast.

Summary

Neither changes in the apparent viscosity of the medium caused by added colloidal materials nor alterations in the viscosity brought about by temperature changes show a linear relationship to the velocity of hydrolysis of sucrose by invertase. Similar behavior of other enzymes may be presumed. It is concluded that the rate of biological enzyme reactions is not directly proportional to the viscosity of the medium.

GENEVA, N. Y. RECEIVED SEPTEMBER 20, 1934

(13) Willstätter *et al.*, *Z. physiol. Chem.*, **115**, 180 (1921); v. Euler, *ibid.*, **105**, 187 (1919); Nelson, *et al.*, *J. gen. phys.*, **15**, 491 (1932); **16**, 571 (1933), etc.

(14) Griffin and Nelson, THIS JOURNAL, **38**, 772 (1916).

[CONTRIBUTION FROM THE GEORGE HERBERT JONES CHEMICAL LABORATORY, UNIVERSITY OF CHICAGO]

A Rapid and Accurate Quantitative Method for the Determination of the Common Carotenoids; Analyses of Beta-Carotene and Leaf Xanthophyll in Thirteen Plant Tissues[1]

BY ELMER S. MILLER[2]

This paper presents a rapid quantitative method for determining carotenoids without separating the components of a plant extract. The spectrophotoelectric method described by Zscheile, Hogness and Young[3] and the analytical procedure devised by Miller[4] were employed in this investigation. The analyses presented in Table III show the accuracy that may be attained by this method. In view of the similarity between the absorption curves of alpha carotene and leaf xanthophyll,[5] it is necessary to separate the carotenes from the xanthophyll before analyses can be made for the respective components. Hence, the writer has made a brief study of the Willstätter and Stoll[6] methanol–ligroin partition method.

Experimental Part

6.4 Mg. of beta carotene and 4.9 mg. of leaf xanthophyll were placed in a flask containing 400 cc. of 89% methanol and 400 cc. of ligroin (b. p. 30–35°). After the carotenoids had dissolved, the solution was transferred carefully to a separatory funnel. The ligroin solution was extracted 5 times with 300-cc. portions of methanol (89% by volume). The last two extractions were colorless. After the ligroin solution had been transferred to a 1-liter volumetric flask and made up to volume, a 10-cc. aliquot portion was placed in a 100-cc. volumetric flask. *In vacuo*, the solution was evaporated almost to dryness (0.5 cc.)—keeping the temperature of the flask below 30° during evaporation. When the vacuum was released, the carotenoids were dissolved in 80 cc. of absolute ethanol and 20 cc. of ether. Analyses of the aliquot portions are given in Table I.

(1) Presented at the Cleveland meeting of the American Chemical Society, Sept. 11, 1934.

(2) National Research Council Fellow.

(3) Zscheile, Hogness and Young, *J. Phys. Chem.*, **38**, 1 (1934).

(4) Miller, *Plant Physiology*, **9**, 693 (1934).

(5) (a) Miller, *Bot. Gaz.*, March issue (1935). (b) Miller, Mackinney and Zscheile, to appear in *J. Biol. Chem.* (1935).

(6) Willstätter and Stoll, "Untersuchungen über Chlorophyll" Berlin, 1913.