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

Note on Invertase Activity in Identical Mixtures in the Liquid and Frozen State[1]

BY Z. I. KERTESZ

The publication of the following experiment was prompted by a recent article by Sizer and Josephson dealing with enzyme[2] kinetics as a function of temperature. These authors confirm the well-known fact that low temperatures do not inactivate the enzymes, lipase, trypsin and invertase, and state that "a sharp break in the relationship of rate to temperature appears at 0 to −2°." This observation is in harmony with and an extension of the writer's findings with invertase activity measured between +40 and −40° and part of which was reported in an article[3] dealing with the velocity of the reaction in undercooled solutions. In connection with this latter work some measurements were performed in identical reaction mixtures of the rate of invertase action in the liquid and solid (frozen) state at the same temperature. These data, heretofore unpublished, gain additional interest by the observations of Sizer and Josephson and throw light on the suspected effect of change of physical state on the velocity of the reaction.

There is little to be added to the experimental technique described in my above article.[3] If reaction mixtures containing sucrose, invertase and buffer in water solution are quickly cooled, temperatures as low as −9° may be reached without freezing the mixture. On the other hand, shaking of the test-tubes during cooling (or sometimes even moving them) is sufficient to cause a rapid solidification of the mixture. Single observations in liquid and frozen mixtures were performed at various temperatures between −2 and −8° but it was at −6.8° that the measurement of the two whole sets of determinations was most successful. These results and a few others indicating the rate of reaction at 20° and at a lower freezing temperature (−17.8°) are given in Table I.

The hydrolysis appears to be much slower in the frozen mixture than in the liquid one. The high value for the first monomolecular constant "*k*" in the frozen mixture at −6.8° is believed to be caused by the higher velocity of the reaction while the mixture was cooled and until it was frozen. The great difference in the velocity in the liquid and frozen state confirms the statement of Sizer and Josephson that the change in phase may be the cause of the break in the rate of enzyme action. It is also apparent that no predictions concerning the rate of a reaction in the frozen state can be made from determinations in liquid mixtures although it is not impossible that in the future some predictable relation may be found between the two factors.

The difference between the rate of hydrolysis in the liquid and frozen mixtures is also apparent in most samples of the lipase series of Sizer and Josephson, although this is not emphasized by these authors.

The reason for the drop in the velocity upon freezing may be the restricted availability of water for the hydrolysis. This may well be the case because previous observations indicated[4] that the amount of water available in invertase reaction mixtures has more effect on the velocity of the reaction than physical conditions as changes in the viscosity, for instance. The possibility of an effect of the freezing on the enzyme itself cannot be disregarded. This effect, if any, must be temporary because when frozen reaction mixtures were melted, they exhibited a normal velocity of hydrolysis.

There is some uncertainty about the significance of results obtained in enzyme reaction mixtures in the presence of glycerol, ethanol and other chemicals used in order to lower the freezing point. In the writer's work difficulties were experienced with these materials in the study of invertase action. They exerted considerable effect on the reaction above the freezing point, as is observable in the lipase hydrolysis data of Sizer and Josephson. The use of these materials would open easy avenues of approach to the problem but their effect on the reaction is definite but not constant (see the lipase table of Sizer and Josephson) and thus there is an element of unreliability about results obtained by their use. The effect of these compounds seems to be again in changing the proportion of water available for the hydroly-

(1) Article III on "Water relations of enzymes." Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Article No. 466, August 15, 1942.

(2) I. W. Sizer and E. S. Josephson, *Food Research*, **7**, 200 (1942).

(3) Z. I. Kertesz, *Z. physiol. Chem.*, **216**, 229 (1933).

(4) Z. I. Kertesz, THIS JOURNAL, **57**, 345 (1935).

TABLE I

INFLUENCE OF TEMPERATURE AND CONDITION ON THE VELOCITY OF SUCROSE HYDROLYSIS BY INVERTASE

Reaction temp., °C.	20.2		−6.8		−6.8		−17.8	
Condition	Liquid		Liquid		Frozen		Frozen	
	min.	$k \times 10^5$	min.	$k \times 10^5$	min.	$k \times 10^5$	min.	$k \times 10^5$
	60	259.6	120	38.30	233	(35.80)	2,820	0.265
			410	39.62			7,200	.388
	120	255.6	1038	36.35	1038	10.54	10,080	.372
			1215	36.70	1218	10.62	17,280	.404
	240	273.8	1218	37.45	1563	10.32	20,160	.393
			1395	40.35			27,350	.201
	360	222.8	1740	40.60			28,970	.221
	Av.	253.0		38.5		10.49		.321

sis rather than by altering the physical characteristics of the solutions.

Summary

The velocity of invertase action in a frozen mixture at −6.8° was only 27% of that in a like mixture in the liquid state. The diminished availability of water may be responsible for this phenomenon.

GENEVA, N. Y. RECEIVED AUGUST 20, 1942

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE STATE COLLEGE OF WASHINGTON]

Some Derivatives of 2-Propionyl-1-naphthol

BY C. M. BREWSTER AND G. G. WATTERS

Since the substitution of acyl and alkyl groups for nuclear hydrogen in phenols has given compounds with marked germicidal properties, we have extended our study in this field by preparing some derivatives of 2-propionyl-1-naphthol, with changes in side chain and nucleus.

We have compared methods of preparation of 2-propionyl-1-naphthol[1] and found little difference in yield, whether the process is carried out directly with α-naphthol or by intra-molecular rearrangement of the ester first formed through the action of the anhydride. However, the direct method requires fewer steps and we have been able to minimize the formation of purple by-products by the procedure given in the experimental part.

In attempting to prepare a di-acyl naphthol from 2-acetyl-1-naphthol by condensation with propionic acid, using zinc chloride as condensing agent, we obtained 2-propionyl-1-naphthol in good yield, the larger acyl group replacing the smaller. A similar replacement occurred when benzoic acid reacted with 2-acetyl-1-naphthol; however, the yield was very small. This replacement recalls the method of preparation of higher members of the salol series, by heating salol with eugenol or other phenols; the higher phenol replaces the lower.[2]

When examining some crystals of 2-propionyl-1-naphthol in subdued light, it was accidentally discovered that the compound shows marked triboluminescence, and this phenomenon persists whether the compound is dry or moistened with water or ethanol, even after removal of traces of impurities.

Reduction of acyl naphthols replaced the carbonyl oxygen by hydrogen and resulted in alkyl naphthols which showed an increase in germicidal activity. However, the pure reduced compounds on standing in the air slowly turned to brown oils and their preservation was difficult. It was anticipated that modification of the acyl side-chain might give compounds of greater stability, and such compounds have been made by condensation of the acyl group with aldehydes.[3] While there was marked reactivity with 2-propionyl-1-naphthol, we have been able to isolate only two derivatives in pure form. Acid condensing agents such as zinc chloride, aluminum chloride, or concentrated sulfuric acid produced marked color

(1) Witt and Braun, *Ber.*, **47**, 3216 (1914); Fries, *ibid.*, **54**, 709 (1921); Stoughton, THIS JOURNAL, **57**, 204 (1935).

(2) German Patent 111,656; also May and Dyson, "The Chemistry of Synthetic Drugs," 4th edition, 1939, p. 219.

(3) Kostanecki, *Ber.*, **31**, 705 (1898); Pfeiffer, Kalckbrenner and Levin, *J. prakt. Chem.*, **119**, 109 (1928); Cheema, Gulati and Venkataraman, *J. Chem. Soc.*, 930 (1932).