and G. Menschikoff[3] reported: specific gravity $^{20}_{20}$ 1.0455; n^{20}_{D} 1.5430; $[\alpha]^{20}_{D}$ $-82.20°$.

Vapor Pressure of Anabasine.—The vapor pressure of the purified anabasine was determined by the same method used in previous work on vapor pressure of fumigants.[4]

Table I shows the vapor pressures obtained at a number of temperatures from 79.0 to 280.6°. The boiling point was found to be 280.9° compared with 276.0° reported by Oréchoff and Menschikoff[3] and 280–282° reported by C. R. Smith.[5] The vapor pressure can be represented with an accuracy of about 5% up to 100° and to better than 0.10% for the remainder of the temperature range investigated by the empirical equation $\log P_{\text{mm.}} = 7.2423 - (2416.14/T_{\text{K}})$.

Optical Rotation of Anabasine.—The optical rotation was determined at 20°, and for the pure material it was found to be $[\alpha]^{20}_{D}$ $-59.66°$.

TABLE I

VAPOR PRESSURE OF ANABASINE

Temp., °C.	Press. obs., mm.	Temp., °C.	Press. obs., mm.
79.0	2.5	218.6	211.5
100.0	6.0	280.6	756.8
137.1	31.6	280.9	760.0
184.2	85.4	282.4	776.1

Optically active anabasine sulfate has been found to racemize by heating in a sealed tube. Menschikoff, Gregorovitch and Oréchoff[6] observed that when a dilute solution of the sulfate had been heated in a sealed tube at 200° for seventy hours and the anabasine freed, the rotation had changed to $[\alpha]^{20}_{D}$ $-25°$ from presumably $[\alpha]^{20}_{D}$ 82.20°, while after heating for 120 hours $[\alpha]^{20}_{D}$ was $-3.0°$. About ten months after the rotation of $-59.66°$ was measured another sample was purified but this showed a rotation of $[\alpha]^{20}_{D}$ $-51.93°$, thereby indicating auto-racemization at room temperature. Further work is being done on this constant, and will be reported in a subsequent contribution.

Summary

Anabasine was carefully purified and the following constants determined: specific gravity, refractive index, optical rotation, vapor pressures at different temperatures, and its boiling point. An equation was obtained that represents the change of vapor pressure with change in temperature to a high degree of accuracy.

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(3) A. Oréchoff and G. Menschikoff, *Ber.*, **64**, 266 (1931).

(4) O. A. Nelson, *Ind. Eng. Chem.*, **20**, 1380 (1928).

(5) C. R. Smith, THIS JOURNAL, **53**, 277 (1931).

(6) G. Menschikoff, Gregorovitch and A. Oréchoff, *Ber.*, **65**, 1126 (1932).

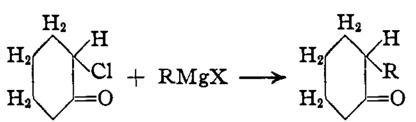
WASHINGTON, D. C. RECEIVED JUNE 13, 1934

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

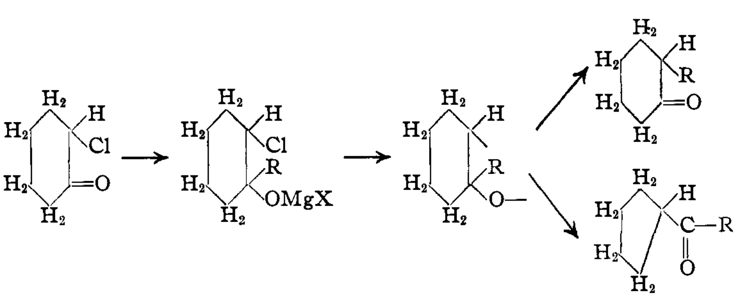
Cis- and *Trans*-Chlorohydrins of Δ^1-Methylcyclohexene[1]

BY PAUL D. BARTLETT AND ROBERT H. ROSENWALD

The reaction of Grignard reagents with 2-chlorocyclohexanone was reported by Bouveault and Chereau[2] to take the following course:



In a very recent paper, Tiffeneau and Tchoubar[3] have shown that this reaction represents a special case of the behavior established years ago for chloroacetone[4] with phenylmagnesium bromide. The first step in the reaction is normal addition of the Grignard reagent to the carbonyl group, and the resulting magnesium derivative of a chlorohydrin is rearranged, by heating, into a 2-alkylcyclohexanone and a smaller amount of an alkyl cyclopentyl ketone. As formulated by Tiffeneau the reactions are



In investigating the mechanism of this reaction shortly before the appearance of the paper by Tiffeneau and Tchoubar, we had obtained results similar to theirs. The isolation of the chlorohydrin (I) in yields up to 82%, from the reaction of 2-chlorocyclohexanone with methylmagnesium bromide, and the isolation of the corresponding bromohydrin from the reaction using 2-bromo-

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[1] From a thesis presented by Robert H. Rosenwald in partial fulfilment of the requirements for the degree of Master of Science at Minnesota, 1934.

(2) Bouveault and Chereau, *Compt. rend.*, **142**, 1986 (1906).

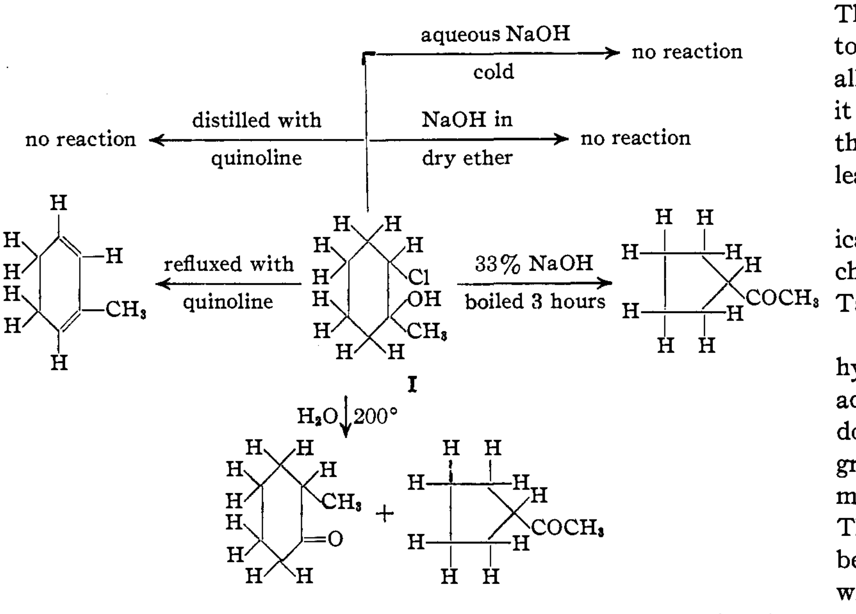
(3) Tiffeneau and Tchoubar, *ibid.*, **198**, 941 (1934).

(4) Tiffeneau, *Ann. chim.*, [8] **10**, 367 (1907).

cyclohexanone, convinced us that the reaction of Bouveault and Chereau involved normal addition of the Grignard reagent to the carbonyl group as the first step, even when the more reactive bromine atom is present in the alpha-position to the carbonyl group.

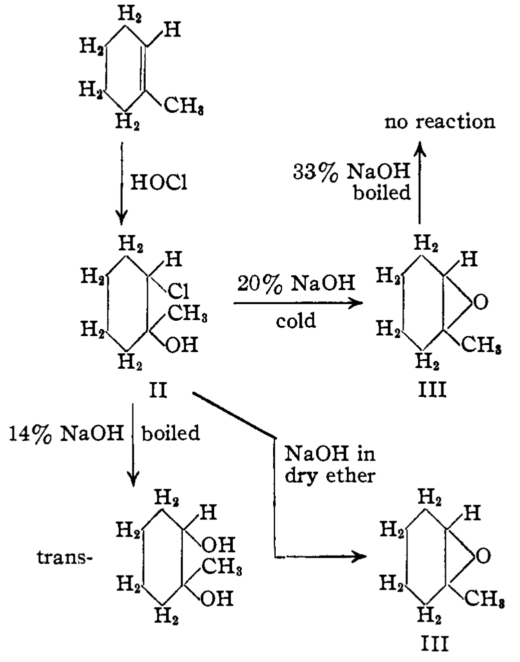
Further investigation of the chlorohydrin (I) revealed that its chemical properties were unusual. It is characteristic of the previously known chlorohydrins of the cyclohexane series—made by adding hypochlorous acid to the corresponding cyclohexenes—that dilute aqueous sodium hydroxide, or powdered sodium hydroxide in dry ether, converts them rapidly at room temperature into the corresponding cyclohexene oxides, with removal of hydrogen chloride.

This is not the case with the chlorohydrin produced from methylmagnesium bromide and 2-chlorocyclohexanone. This chlorohydrin is unaffected by such moderate alkaline treatment, while refluxing with 33% sodium hydroxide converts it into methyl cyclopentyl ketone as the sole product. The following outline shows the reactions of this chlorohydrin



In no case was any oxide obtained from this chlorohydrin.

Suspecting that the chlorohydrin I was an isomer of the one formed when hypochlorous acid is added to methylcyclohexene, we carried out the latter reaction and obtained a chlorohydrin (II) whose chemical properties were entirely different from those of the product (I) from the Grignard reaction



The oxide (III) was obtained from this chlorohydrin with great ease. It is a liquid of characteristic odor, which boils at 135° at 740 mm. This oxide is completely stable toward even hot concentrated alkali, which fact eliminates it as an intermediate in any of the alkaline transformations leading to other products.

The slight difference in physical properties between the two chlorohydrins is shown in Table I.

In all known cases in which hypochlorous acid has been added to a carbon-to-carbon double bond, the hydroxyl group has appeared on the more alkylated carbon atom. This was established in a number of cases by Detoeuf[5] and with especial care for the case, closely related to ours, of Δ^3-*p*-menthene by Kötz

TABLE I

PHYSICAL PROPERTIES OF ISOMERIC 1-METHYL-2-CHLOROCYCLOHEXANOLS

	Isomer	B. p., °C.	Mm.	d_{25}	n_D^{25}
I	(from Grignard)	73–75	15	1.0995	1.4775
II	(from HOCl)	90–95	28	1.1006	1.4850

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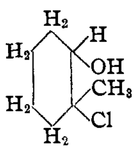
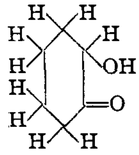
[5] A. Detoeuf, *Bull. soc. chim.*, [4] **31**, 169–176 and 176–181 (1922).

and Busch.[6] Since the reactions of our chlorohydrin (II) are those of the known chlorohydrins, it would be entirely unwarranted to assume the orientation (IV) to explain why its reactions differ from those of the new compound (I). The isomerism is evidently geometrical, a conclusion borne out by the existence of similar isomers of the unsubstituted 2-chlorocyclohexanol.[7]



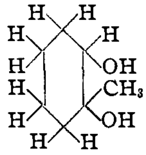
IV

Several questions are raised by these reactions, to which a final answer cannot now be given. The geometrically simple assumption that hypochlorous acid adds *cis* to the double bond, and that the *cis* chlorohydrin is the one that forms an oxide, can by no means be accepted without question. *Trans*-additions to a double bond are very common, and Walden inversion during the replacement of a halogen atom is not improbable. To get an idea of the kind of orientation to be expected during the Grignard addition, we added methylmagnesium bromide to 2-hydroxycyclohexanone (V). The product was the known *cis*-1-methylcyclohexane-diol-1,2 (VI) identified by its melting point and by the formation of a boric acid addition compound.[8] If the directing influence of a hydroxyl group may be considered identical with that of a halogen atom, then this experiment points to *trans*-addition of hypochlorous acid to the double bond. Further work on this question is in progress.



V

cis-



VI

Experimental Part

2-Chlorocyclohexanone was at first prepared by the method of F. Ebel,[9] passing chlorine into a mixture of water and cyclohexanone in the presence of powdered calcium carbonate. The yields were about 50% of product boiling at 82–85° under 14 mm. pressure. Direct chlorination of cyclohexanone in glacial acetic acid was found to be a more efficient procedure. Excess ketone is used. By this method no high-boiling residue results, and in a series of runs a quantitative yield can be approached by using the solvent and excess ketone over and over. A run was carried out as follows: 75 g. of cyclohexanone and 225 cc. of glacial acetic acid were placed in a 500-cc. long-necked flask fastened to a shaker and immersed in an ice-bath. The flask was connected through a three-way stopcock to suction and to a 2-liter gasometer containing chlorine. The flask was exhausted at the beginning of the run and after the introduction of each two liters of gas, to cut down the back vapor pressure of hydrogen chloride. The chlorine was absorbed very rapidly (two liters in less than a minute). After the introduction of 14 liters of chlorine, the product was at once distilled under diminished pressure, and all distillates below the main fraction (82–85°, 14 mm.) were returned to the chlorination flask and used for another run.

Methyl bromide was prepared by the method of H. Bygdén,[10] converted at once into the Grignard reagent, and the standardized solution kept in paraffin-sealed bottles.

1-Methyl-2-chlorocyclohexanol (I).—100 cc. of the standard Grignard solution (1.32 molar) was added slowly with stirring to an equivalent amount (17.3 g.) of 2-chlorocyclohexanone dissolved in 100 cc. of dry ether, and surrounded by an ice-bath. The product was hydrolyzed immediately with iced 10% sulfuric acid. The ether solution, after washing with sodium carbonate solution and drying with sodium sulfate, was distilled under diminished pressure. Yields varied from 55 to 82% of a product boiling at 69–74° under 12 mm. pressure. The 82% yield resulted from a run of four times the amounts described here.

The pure chlorohydrin has a boiling point of 73–75° at 15 mm., n^{25}_{D} 1.4775, d_{25} 1.0995. Tiffeneau and Tchoubar[3] give b. p. 75–76° (14 mm.), n^{19}_{D} 1.4811, d_{19} 1.0934.

Anal. Calcd. for $C_7H_{13}OCl$: Cl, 23.87. Found: Cl, 23.45.

2-Bromocyclohexanone was prepared according to the directions of Kötz.[11] The boiling point was 85–95° (14 mm.).

1-Methyl-2-bromocyclohexanol was prepared in the same manner as the corresponding chloro compound. It had the following properties: b. p. 83° (7 mm.); d_{25} 1.360; n^{27}_{D} 1.5032.

2-Methylcyclohexanone.—In preparing this compound by the Grignard reaction 69 g. of 2-chlorocyclohexanone and an equivalent amount of methylmagnesium bromide were added together as in the preparation of the chlorohydrin (II), and the ether then distilled off and replaced by dry benzene. After refluxing for six hours (temperature 75°) the solution was washed with water and distilled at ordinary pressure. After two redistillations a fraction was obtained boiling from 155–164° and weighing 25 g., corresponding to a 43% yield. The product turned dark on standing. The identification of the ketones present was made by fractional semicarbazone formation, as used by Vavon and Mitchovitch,[12] the separation depending upon the greater rate of reaction of methylcyclohexanone than of methyl cyclopentyl ketone with semicarbazide. Of three semicarbazone fractions prepared by successive treatment of the same ketone sample with insufficient semicarbazide, the first two yielded on recrystallization only the semicarbazone, m. p. 194°, of 2-methylcyclohexanone. The third fraction, recrystal-

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(6) Kötz and Busch, *J. prakt. Chem.*, **119**, 1 (1928).

(7) This will be the subject of a later communication.

(8) C. J. Maan, *Rec. trav. chim.*, **48**, 342 (1929).

(9) F. Ebel, *Helv. Chim. Acta*, **12**, 9 (1929).

(10) H. Bygdén, *J. prakt. Chem.*, **104**, 286 (1922).

(11) Kötz, *Ann.*, **358**, 195 (1907).

(12) Vavon and Mitchovitch, *Bull. soc. chim.*, [4] **45**, 965 (1929).

lized from a water–alcohol mixture, showed a melting point of 130–132°. By recrystallization from water, pure methylcyclopentyl ketone semicarbazone, m. p. 146–147°, was obtained and identified by a mixed melting point with a synthesized sample. The melting point of this semicarbazone depends upon its state of subdivision; similarly ground samples of the isolated and synthesized semicarbazones melted separately at 143–145°, mixed at 143–144°. The relative amounts of the semicarbazones melting at 194° and at 146–147° were 10 to 1 in the product of the Grignard reaction.

Methylcyclopentyl ketone was synthesized by the dichromate oxidation of methylcyclopentylcarbinol, which was prepared from cyclopentylmagnesium bromide and acetaldehyde.

Distillation of Chlorohydrin (I) with Quinoline.—Ten grams of 2-chloro-1-methylcyclohexanol (I) and 20 cc. of quinoline were distilled rapidly at ordinary pressure and the distillate collected up to 220°. The distillate was shaken with dilute hydrochloric acid and 7 g. of the chlorohydrin (I) recovered by distillation, b. p. 73–75° (15 mm.).

This 7 g. of chlorohydrin was refluxed for an hour with 14 cc. of quinoline. The solution turned dark red and visible dehydration occurred. Distillation left a considerable gummy residue. The layers of the distillate were separated, the upper layer dried with calcium chloride and distilled through a special small flask with a vacuum-jacketed column; 1.1 g. of distillate was obtained having b. p. 108–110° and n^{26}_{D} 1.4680. Zelinsky and Gorsky[13] reported for a similar sample prepared by refluxing 1-methyl-1,2-dibromocyclohexane with quinoline, b. p. 110° and n^{20}_{D} 1.4710.

Attempts to Prepare an Oxide from the Chlorohydrin (I).—Thirteen grams of 2-chloro-1-methylcyclohexanol was placed in a 150-cc. Florence flask with 3.6 g. of sodium hydroxide dissolved in 20 cc. of water. The flask was shaken for one and one-quarter hours on the shaker, a procedure sufficient to convert 2-chlorocyclohexanol completely into cyclohexene oxide. The chlorohydrin in this case was recovered unchanged. Next 14 g. of the chlorohydrin (I) was dissolved in 100 cc. of dry ether and shaken for seven hours with 20 g. of powdered sodium hydroxide; 10 g. of the original chlorohydrin was obtained, leaving a residue of 1.5 g. on distillation.

Methylcyclopentyl Ketone.—The rearrangement of the chlorohydrin (I) was best accomplished by refluxing small (10-cc.) portions of the chlorohydrin with six times their volume of 33% sodium hydroxide solution for three hours. The product, obtained in about 50% yield, boiled at 160–165° and gave a semicarbazone melting at 146–147°. The ketone was identified by mixed melting point of its semicarbazone and by reduction to the secondary alcohol, whose phenylurethan melts at 69.5–70°. The factor reducing the yield is that alkali strong enough to rearrange the chlorohydrin is also able to condense the ketone.

Rearrangement of the Chlorohydrin (I) with Water at 200°.—Fifteen grams of the chlorohydrin and 15 g. of water were sealed in a Carius tube and heated to 200° for six hours. Besides a small amount of a high-boiling fraction and a considerable amount of residue, a ketone fraction of 2.4 g. was obtained boiling at 42–46° at 14 mm. from which semicarbazones of m. p. 144–145° and 188–189.5° were obtained.

Δ^1-Methylcyclohexene was prepared in 64% yield by adding methylmagnesium bromide to 80 g. of cyclohexanone in the usual manner and adding a few crystals of iodine to the ethereal extract containing the 1-methylcyclohexanol prior to distillation. This was then distilled under atmospheric pressure, when water and the hydrocarbon distilled over together. The product, on redistillation, boiled at 108–111°. Hydroquinone was added immediately to the 50 g. of product as an antioxidant, to guard against possible abnormal addition of hypochlorous acid due to "peroxide effect."

2-Chloro-1-methylcyclohexanol (II) was prepared by the method described in "Organic Syntheses" for 2-chlorocyclohexanol,[14] except that ether extraction was used to isolate the product. From 50 g. of Δ^1-methylcyclohexene, fractional distillation under 28 mm. pressure yielded 10 g. of b. p. below 90°, consisting mainly of 1-methyl-1,2-epoxycyclohexane; 21.7 g., b. p. 90–95°; 7.3 g., b. p. 95–125°; and 5.5 g. of residue. The fraction boiling from 90 to 95° at 28 mm. pressure was used in the experiments. It had a density of 1.1006 at 26° and a refractive index of 1.4850 at 25°.

1-Methyl-1,2-epoxycyclohexane.—5.5 grams of the chlorohydrin (II) was shaken at room temperature for an hour and a half with a solution of 2 g. (one-third excess) of sodium hydroxide in 10 cc. of water. The upper layer was separated, washed with water and distilled. The product was 2.3 g. boiling at 135–140°; 0.5 g. boiling from 140–180°; and 0.5 g. boiling from 180–190°, at 740 mm. pressure. The main fraction represented a 55% yield of 1-methyl-1,2-epoxycyclohexane, having n^{29}_{D} 1.4320. This oxide is described by Nametkin and Jarzeff.[15] It was unaffected by three hours of refluxing with 33% sodium hydroxide.

Hydrolysis of the Chlorohydrin (II).—3.2 grams of the chlorohydrin was refluxed for three hours with 3.5 g. of sodium hydroxide in 25 cc. of water. The product was extracted with ether. Distillation under 25 mm. pressure yielded 1.3 g. of a heavy liquid boiling at 130° at this pressure. On cooling this crystallized. Recrystallization from ether–benzene solution gave a product melting at 78–81°. The melting point of the pure *trans*-form of 1-methylcyclohexanediol-1,2 is 85°; that of the *cis*-form is 68°.[8]

Anal. Calcd. for $C_7H_{14}O_2$: C, 64.6; H, 10.8. Found: C, 64.1; H, 10.7.

Addition of Methylmagnesium Bromide to 2-Hydroxycyclohexanone.—Ten grams of 2-hydroxycyclohexanone (m. p. 110–118°) suspended in ether, was added to two equivalents of methylmagnesium bromide, the temperature being kept below 8° during the entire addition. After hydrolysis of the product by pouring on ice, fifteen extractions of the aqueous solution with chloroform were made. Evaporation of the chloroform yielded 3.7 g. of a heavy liquid which, after vacuum distillation, crystallized. It was recrystallized from ether-benzene solution to a

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[13] Zelinsky and Gorsky, *Ber.*, **41**, 2630 (1908).

[14] "Organic Syntheses," Collective Volume I, 1931, p. 151.

[15] Nametkin and Jarzeff, *Ber.*, **56**, 1803 (1923).

melting point of 63–66°, but could not be brought to the melting point (68°)[8] of pure *cis*-1-methylcyclohexanediol-1,2. Its configuration was checked by the preparation of a sodium borate complex, of which 1.57 g. was obtained from 1 g. of the diol, following the procedure of Maan.

Summary

The chlorohydrin formed by addition of methylmagnesium bromide to 2-chlorocyclohexanone is a geometrical isomer of the one made by addition of hypochlorous acid to 1-methylcyclohexene; whereas the latter reacts with aqueous alkali at room temperature to give methylcyclohexene oxide, its isomer is unaffected by this treatment, but is converted by hot alkali into methylcyclopentyl ketone.

MINNEAPOLIS, MINNESOTA RECEIVED JUNE 13, 1934

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

The Influence of Native Proteins on the Activity of Yeast Invertase

BY J. M. NELSON AND EVERETT L. SAUL

While examining the influence of varying hydrogen-ion concentration on the activity of yeast invertase in the presence of normal and anti-invertase sera, obtained from rabbits, Mrs. Schubert[1] noticed that on the acid side of the enzyme's optimum *p*H (4.5–5.0) both sera caused an increase in the rate of hydrolysis of sucrose. She also found that egg albumin exerts a similar influence under these conditions. Fine[2] reported that the presence of high concentrations of blood serum increases the activity of yeast invertase in the *p*H ranges 3–5 and 7–8. Increases in enzymic activity, due to the presence of added proteins, has been observed also in the case of lipases.[3,4,5]

The results given in the present paper were obtained in continuing the study of the influence exerted by proteins on the activity of yeast invertase preparations. As a brief summary, it may be stated that not only the purity (degree of activity of the enzyme preparations), and the *p*H, but also the condition of the protein, native or denatured, were found to be factors in determining the influence of the latter on the enzyme's activity. The procedure followed was to compare the rates of hydrolysis of sucrose in the presence and absence of added protein. Egg albumin, pepsin, edestin, serum, globin and gelatin were used, and when possible in the crystalline form, in order to minimize as much as possible any effect due to extraneous matter. Increases in the activity of the enzyme were observed, provided the *p*H of the hydrolyzing sucrose solutions were less than 4.5, the protein added being in the native condition,

TABLE I

DATA SHOWING THE INCREASE IN ACTIVITY OF YEAST INVERTASE IN THE PRESENCE OF NATIVE PROTEIN AT *p*H 3

Twenty-five cc. of highly active invertase preparation RaaDKDADADSD (time value 0.35 minute at 25°) was added to 200 cc. of sucrose solution, containing added protein as indicated below. Final concentration of sucrose 10%, *p*H 3, citrate–phosphate buffer about 0.15 *M*, temperature of hydrolysis 25°.

	Time in minutes	Change in rotation in degrees
A. 20 mg. of native edestin per 225 cc. of solution		
	2	..
	19	0.28
	27	.40
	45	.70
	57	.88
	Mean rate[a]	.0162
B. 10 mg. of native egg albumin per 225 cc. of solution		
	2	..
	25	0.33
	35	.47
	45	.62
	57	.78
	82	1.15
	Mean rate	0.0143
C.	2	..
	30	0.35
	52	.60
	72	.87
	91	1.09
	109	1.32
	Mean rate	0.0124

Increased rate of A over C = 30%. Increased rate of B over C = 15%. Similar increases in the enzyme's activity were observed when ox-serum, native pepsin and native globin were used.

[a] Rate = change in rotation per minute, obtained by dividing change in rotation by the corresponding time interval.

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(1) Marcelle V. Schubert, "Dissertation," Columbia University, 1933.

(2) J. Fine, *Biochem. J.*, **24**, 1282 (1930).

(3) O. Rosenheim and J. A. Shaw-Mackenzie, *J. Physiol.*, **40**, xii (1910).

(4) B. S. Platt and E. R. Dawson, *Biochem. J.*, **19**, 869 (1925).

(5) K. G. Falk, *J. Biol. Chem.*, **96**, 53 (1932).