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The Behavior of Glucose Dimethylacetal toward Carbohydrases[1]

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Under the title "The Enzymotic Cleavage of Acetals," Neuberg and Ziffer[2] have reported that acetal itself (acetaldehyde diethylacetal) is subject to enzymotic hydrolysis by top yeast, by taka-diastase, and by commercial emulsin, in aqueous solution at 37° in the presence of calcium carbonate and toluene. Upon the appearance of that paper, our own experiments, which were then in progress, on the behavior of glucose dimethylacetal[3] toward various carbohydrases were extended to include the conditions used by Neuberg and Ziffer. In no case, however, have we been able to detect any cleavage of glucose dimethylacetal by top yeast, taka-diastase, emulsin, maltase, invertase, pancreatic amylase, or malt amylase. The conditions of temperature, time, *p*H, and the final rotation of the solutions are recorded in Table I; the details of the enzyme preparations, amounts, and activities are reported in the Experimental section.

TABLE I

GLUCOSE DIMETHYLACETAL AND CARBOHYDRASES

Enzyme	*p*H	Buffer	Time, hrs.	Temp., °C.	$[\alpha]^{20}D$[a]
Invertase	7.1	Phosphate[b]	20	20	+14.2°
Invertase	5.1	Phosphate	20	20	15.3
Maltase	6.9	Phosphate	21	20	14.0
Emulsin	5.4	Acetate[c]	68	20	14.7
Malt amylase	4.6	Acetate	65	20	14.3
Pancreatic amylase	7.1	Phosphate[d]	22	20	14.2
Taka-diastase	5.2	Phosphate	20	20	14.4
Taka-diastase	7.1	Phosphate	20	20	14.1
"Clarase"	5.3	Phosphate	23	20	14.0
Taka-diastase (concd.)	5.5	Acetate	24	20	13.7
Taka-diastase (concd.)	[e]	[e]	24	38	14.6
Emulsin	[e]	[e]	24	38	15.6
Top yeast	[e]	[e]	45	38	14.2

[a] The specific rotation of the glucose dimethylacetal was +14.3 ± 0.3° in water (*c*, 5); if completely hydrolyzed to glucose its apparent specific rotation would be +41.8°. All other specific rotations are correct to ±0.9°. [b] The phosphate buffers are about 1% in sodium phosphates. [c] The acetate buffers are about 0.1 or 0.2 *N* in acetic acid–sodium acetate mixtures. [d] The solution was also 0.04 *M* in sodium chloride. [e] The aqueous solution contained no buffer except solid calcium carbonate; two drops of toluene were added.

Our results are in accord with the statement by Pacsu[4] that fructose dimethylacetal is not affected by yeast at *p*H 7, or by invertase at *p*H 4 or 7. The failure of these glucose and fructose acetals to be cleaved by enzymes, however, must not be construed as evidence that other acetals may not be susceptible to enzymotic hydrolysis.

Experimental

Cleavage Experiments.—A weighed amount of glucose dimethylacetal[3] in a volumetric flask, such that the final concentration would be 2 g. per 100 cc., was dissolved in the desired buffer solution, a definite weight or volume of the enzyme added, and the flask filled to the mark with additional buffer solution. After standing for the length of time and at the temperature noted in Table I, the solution was adjusted to volume and filtered if necessary, and the rotation observed in a 1-dm. tube at 20°.

Invertase.—An autolysate of bakers' yeast was dialyzed, and treated with bentonite as described previously.[5] The eluate was concentrated and purified further by precipitation with ammonium sulfate; the aqueous extract, after dialysis, contained 32.6 invertase units[6] in 100 cc., with a time value[6] of 0.20 minute. For the cleavage experiments, 0.5 g. of the acetal, in the desired buffer solution, was treated with 1 cc. of the invertase solution in a total volume of 25 cc.

Maltase.—The fractional autolysis of brewers' yeast according to Weidenhagen[7] produced a maltase solution of concentration such that 1.25 g. of maltose in a total volume of 25 cc., containing 1% phosphate buffer of *p*H 6.9, was 50% hydrolyzed by 5 cc. of the enzyme solution in one hundred and eighty-seven minutes at 20°. The enzyme solution was capable also of hydrolyzing α-methylglucoside. For the cleavage experiment, 5 cc. of the maltase solution was used with 0.5 g. of the acetal.

Emulsin.—Sweet almond emulsin was obtained by the zinc sulfate and tannic acid procedure of Helferich and his collaborators.[8] The activity of the enzyme was such that 25 cc. of a 2% solution of salicin in about 0.2 *N* sodium acetate buffer of *p*H 5.0 was 50% hydrolyzed by 1 cc. of emulsin solution in ten and two-tenths minutes at 20°. For the cleavage experiments 1 cc. of emulsin was added to 0.5 g. of the acetal.

Malt Amylase.—The aqueous extract from distillers' barley malt was purified by the method of Sherman, Caldwell and Doebbeling.[9] The enzyme preparation had an activity of 1240 on the scale of Sherman, Kendall and Clark[10]; this is a measure of the saccharogenic rather than

(1) Publication authorized by the Surgeon General, U. S. Public Health Service.

(2) Neuberg and Ziffer, *Enzymologia*, **5**, 389 (1939).

(3) Wolfrom and Waisbrot, THIS JOURNAL, **60**, 855 (1938).

(4) Pacsu, THIS JOURNAL, **60**, 2278 (1938).

(5) Adams and Hudson, *ibid.*, **60**, 982 (1938).

(6) C. Oppenheimer, "Die Fermente und ihre Wirkungen," fifth edition, 1928, Vol. III, pp. 770–774.

(7) Weidenhagen, *Z. Ver. deut. Zuckerind.*, **80**, Tech. Tl. 157 (1930).

(8) Helferich, Winkler, Gootz, Peters and Günther, *Z. physiol. Chem.*, **208**, 95 (1932).

(9) Sherman, Caldwell and Doebbeling, *J. Biol. Chem.*, **104**, 501 (1934).

(10) Sherman, Kendall and Clark, *J. Biol. Chem.*, **32**, 1073 (1910).

of the amyloclastic power. No cleavage was observed when 10 mg. of enzyme was added to 0.2 g. of the acetal.

Pancreatic Amylase.—The method of purification of Sherman and Schlesinger[11] yielded a product with a saccharogenic activity[10] of 3300. Of this amylase, 6 mg. was without effect on 0.5 g. of the acetal.

Taka-diastase.—This was a commercial preparation with a saccharogenic activity of 10.

"Clarase."—This was a more concentrated commercial preparation of the amylase from *Aspergillus oryzae*, and had a saccharogenic activity of 28. With each of these last two preparations 15 mg. was added to 0.5 g. of the acetal.

Taka-diastase, Concentrated.—Commercial taka-diastase was purified to a saccharogenic activity of 390 by the method of Sherman and Tanberg[12]; it had no action when 10 mg. was added to 0.2 g. of the acetal.

Top Yeast.—To 0.5 g. of the acetal was added 100 mg. of washed top yeast, from the Gunther Brewing Co. of Baltimore, Maryland.

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Summary

Glucose dimethylacetal was not hydrolyzed, under a variety of conditions, by invertase, maltase, emulsin, malt amylase, pancreatic amylase, taka-diastase, or top yeast.

(11) Sherman and Schlesinger, THIS JOURNAL, **34**, 1104 (1912).

(12) Sherman and Tanberg, *ibid.*, **38**, 1638 (1916).

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The Cleavage of Cellobiose and Celtrobiose by Emulsin[1]

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In a recent paper by Helferich and Pigman[2] it was reported that the β-galactosido linkage of neolactose (4-β-D-galactosido-D-altrose)[3] was cleaved by sweet almond emulsin only one-seventh as rapidly as the similar linkage of lactose (4-β-D-galactosido-D-glucose). The difference in the ease of hydrolysis of the two β-galactosides was attributed to the change in configuration of the third carbon atom of the glucose molecule which occurs (together with an inversion about the second carbon atom) during the transformation of lactose into neolactose; the effect was likened to that of ortho-substitution, contrasted to that of meta- or para-substitution, in the hydrolysis of phenylglucosides by sweet almond emulsin.[4]

With the corresponding β-glucosides, cellobiose (4-β-D-glucosido-D-glucose) and celtrobiose (4-β-D-glucosido-D-altrose),[5] available in this Laboratory, we decided to make a similar comparison of their behavior toward sweet almond emulsin. The cleavage of cellobiose by emulsin was observed first by Fischer and Zemplén,[6] and has been studied more recently by Weidenhagen[7] and by Helferich, Gootz and Sparmberg.[8]

In Tables I and II are recorded the data obtained in our comparisons of the action of emulsin upon the two disaccharides. The hydrolysis constants are based on the assumption that the reactions are unimolecular. The ratio of the average value of the constants, 10.8:1.59, indicates that cellobiose is hydrolyzed 6.8 times more rapidly than celtrobiose under the same conditions. The lengths of time required for 50% hydrolysis of the disaccharides, as obtained by interpolation, are 281 and 2105 minutes for cellobiose and celtrobiose, respectively; their ratio indicates that cellobiose is hydrolyzed 7.5 times more rapidly than celtrobiose. For 25% hydrolysis, a quotient of 6.7 is obtained. Thus it appears that the inversion of H and OH which is brought about on the second and especially on the third carbon atoms of the reducing glucose molecule in the transformation of cellobiose to celtrobiose has the same effect upon the rates of hydrolysis of these disaccharides as does the similar change of configuration in the corresponding β-galactosides lactose and neolactose. The

(1) Publication authorized by the Surgeon General, U. S. Public Health Service.

(2) Helferich and Pigman, *Ber.*, **72**, 212 (1939).

(3) Richtmyer and Hudson, THIS JOURNAL, **57**, 1716 (1935).

(4) Helferich and Scheiber, *Z. physiol. Chem.*, **226**, 272 (1934).

(5) Richtmyer and Hudson, THIS JOURNAL, **58**, 2534 (1936).

(6) Fischer and Zemplén, *Ann.*, **365**, 1 (1909).

(7) Weidenhagen, *Z. Ver. deut. Zuckerind.*, **80**, Tech. Tl. 11 (1930).

(8) Helferich, Gootz and Sparmberg, *Z. physiol. Chem.*, **205**, 201 (1932).