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]

BY NELSON K. RICHTMYER AND C. S. HUDSON

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).

change from D-glucose to D-altrose in the "aglycone" portion of the glycosides, which has resulted in the hydroxyl on carbon atom 3 being nearer spatially to the glycosidic union at carbon atom 4, as interpreted by the customary models, has produced such a steric hindrance to cleavage of the disaccharide linkage at carbon atom 4 that the velocity of hydrolysis has been reduced to one-seventh of its original rate.

The fact that celtrobiose is hydrolyzed by the β-glucosidase of sweet almond emulsin, and is not cleaved by maltase (an α-glucosidase), confirms the assignment of the β-glucosidic linkage in celtrobiose; this linkage had been assumed previously because the sugar was prepared from cellobiose.

TABLE I

THE CLEAVAGE OF CELLOBIOSE BY EMULSIN AT 20° AND *p*H 5.0

| Time, min. | $\alpha^{20}D$ | % cleavage | $K \times 10^4$ |
|---|---|---|---|
| 0 | (+1.68°) | 0 | .. |
| 60 | 1.80 | 12.8 | 9.9 |
| 65 | 1.82 | 14.9 | 10.8 |
| 120 | 1.94 | 26.3 | 11.1 |
| 180 | 2.04 | 37.4 | 11.3 |
| 210 | 2.09 | 42.7 | 11.5 |
| 255 | 2.14 | 47.0 | 10.8 |
| 300 | 2.18 | 52.0 | 10.6 |
| 420 | 2.29 | 63.0 | 10.3 |
| (∞) | (2.65) | (100.0) | .. |
| | | Average | 10.8 |

TABLE II

THE CLEAVAGE OF CELTROBIOSE BY EMULSIN AT 20° AND *p*H 5.0

| Time, min. | $\alpha^{20}D$ | % cleavage | $K \times 10^4$ |
|---|---|---|---|
| 0 | (+0.69°) | 0 | .. |
| 75 | .74 | 3.6 | 2.09 |
| 420 | .91 | 15.2 | 1.70 |
| 1440 | 1.27 | 40.3 | 1.55 |
| 1860 | 1.37 | 47.2 | 1.49 |
| 2850 | 1.55 | 59.0 | 1.36 |
| 3300 | 1.62 | 63.8 | 1.35 |
| (∞) | (2.15) | (100.0) | .. |
| | | Average | 1.59 |

## Experimental

**Emulsin (β-D-Glucosidase)** was prepared[9] by extraction of defatted sweet almond meal with zinc sulfate solution, fractional precipitation of the enzyme with tannic acid, removal of the tannic acid with acetone and extraction of the emulsin from the residue with water. The product had a small negative rotation, necessitating a slight correction in later observations. Although the actual β-glucosidase value was not determined, the activity of the enzyme was such that 25 cc. of a 2% solution of salicin in about 0.2 *N* acetic acid–sodium acetate buffer of *p*H 5.0 was 50% hydrolyzed by 1 cc. of emulsin solution in 10.2 minutes at 20°.

The enzymotic hydrolyses were carried out by dissolving 4.000 g. of cellobiose or 4.211 g. of celtrobiose monohydrate, respectively, in about 0.2 *N* acetic acid–sodium acetate buffer of *p*H 5.0, adding 4 cc. of the emulsin solution at zero time, and diluting with a small additional amount of buffer exactly to 100 cc. at 20°. The cellobiose solution was kept in a water-bath at 20 ± 0.1°, and the celtrobiose solution in a room at 20 ± 1°. At appropriate times, a 15-cc. portion of the desired solution was removed, and 10 cc. of *N* aqueous sodium carbonate added to stop the enzyme action and to complete the mutarotation of the sugars. The optical rotation was observed after five minutes, in a 2-dm. tube at 20°. The data are recorded in Tables I and II, with the percentage cleavage, and the hydrolysis constants calculated in minutes and decimal logarithms.

**Maltase (α-D-Glucosidase)** was prepared by the fractional autolysis of brewers' yeast with ethyl acetate according to Weidenhagen[10]; although the solution was decidedly active toward maltose and α-methyl-D-glucoside, no evidence of cleavage of celtrobiose could be detected at *p*H 7.0 in forty-eight hours at 20°.

One of the authors (N. K. R.) desires to thank the Chemical Foundation of New York for a Research Associateship.

## Summary

Celtrobiose is hydrolyzed by sweet almond emulsin only one-seventh as rapidly as cellobiose. This relationship is in agreement with the experience of Helferich and Pigman with the corresponding galactosides, neolactose and lactose.

The cleavage of celtrobiose by emulsin, but not by maltase, is presented as confirmatory evidence for a β-glucosidic linkage in celtrobiose (4-β-D-glucosido-D-altrose).

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

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