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Determination of the Optical Rotation of β-Fructofuranose by Means of Kinetic Measurements

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The furanose form of fructose which is found in sucrose has been investigated. By means of highly active β-fructofuranosidase sucrose has been hydrolyzed to 99 % in 3 minutes. The reaction has been followed by polarimetric measurements directly on the reacting solution. After complete hydrolysis of the sucrose the optical rotation still changes owing to the sum of the further transformations of α-glucopyranose and of β-fructofuranose. From the sum of the two exponentials the one representing the transformation of β-fructofuranose is evaluated by subtracting the one with the small rate constant due to the glucose. It is shown that the rate constant obtained in this way is identical with that obtained by dissolving crystalline fructopyranose in the same enzyme-buffer solution and measuring the change in optical rotation. The optical rotation of the β-fructofuranose has been found by extrapolating the equation to the time when the concentration of β-fructofuranose is identical with the initial sucrose concentration.

The value of $[\alpha]_{546}^{25}$ is found to be 1.7°. The composition of the equilibrium mixture of furanose and pyranose at 25°C based on the optical rotation of the pyranose and the furanose form and the optical rotation of the equilibrium mixture is 31.56 % furanose and 68.44 % pyranose.

As was pointed out in an earlier publication [1], a valuable contribution to the problem of the enzymatic inversion of sucrose would be the determination of the specific optical rotation of β-fructofuranose. The crystalline form of fructose is the pyranose form and the furanose form has not been crystallized. However, when fructose participates in glycosidic linkage as in the disaccharide sucrose it is present in the furanose form. It is therefore possible to follow the reaction β-fructofuranose → equilibrium mixture after very rapid enzymatic hydrolysis of a fructoside if the contributions from the other degradation products to the optical rotation are known at any given time. A technique of this kind was first described by Armstrong [2]. One of the classical investigations is that of Hudson [3]. He has shown that after a rapid hydrolysis of sucrose by invertase, the fructose mutarotates with the same velocity as crystalline

fructose dissolved in the same medium. From these measurements he obtained $+17°$ for the specific optical rotation of β-fructofuranose, using the sodium line. The identity of the velocity constants for the two opposing reactions is not quite convincing, since that from the sucrose splitting was taken from a part of the curve representing both glucose and fructose transformations. The value given for the optical rotation was based on extrapolation of curved lines and is therefore not very reliable. The same value has later been confirmed by Bailey and Hopkins [4] but also in this case the determination was based on extrapolation of curved lines. Furthermore these authors based their calculations on mutarotation constants taken from the literature without ascertaining that the constants were applicable to their solution containing large amounts of β-fructofuranosidase and other proteins. Since these velocity constants are highly dependent on the concentration of hydrogen ions and on the ionic strength, the constants from the literature are hardly applicable. The calculation of Isbell and Pigmann [5] based on the investigation of Purves and Hudson [6] is very convincing concerning the identity of the velocity constants for both directions of the mutarotation of fructose, but in this work no attempt has been made to evaluate the optical rotation of the furanose form. In the following we shall verify that the five-membered fructose after liberation from its glycoside linkage undergoes one and only one reaction and that this reaction is the transformation to the six-membered fructose. Further we shall derive a value for the optical rotation of the five-membered form.

The reaction can be described in the following way:

$$S \rightarrow G_1 + F_1 \qquad \text{(I)}$$
$$G_1 \rightarrow G_2 \qquad \text{(II)}$$
$$F_1 \rightarrow F_2 \qquad \text{(III)}$$

in which S denotes sucrose, G_1 α-glucopyranose and F_1 β-fructofuranose; G_2 denotes the equilibrium mixture which is formed from the α-glucopyranose and F_2 the equilibrium mixture which is formed from the β-fructofuranose. The velocity constants for the three equations are k_S, k_G, k_F. Assuming that reaction I is instantaneous and that reactions II and III are both first order reactions, the optical rotation α as a function of time for the solution is given by the following:

$$(\alpha_t - \alpha_\infty)_{F+G} = (\alpha_o - \alpha_\infty)_F \exp(-k_F t) + (\alpha_o - \alpha_\infty)_G \exp(-k_G t) \qquad \text{(IV)}$$

Since the value of k_F is approximately ten times that of k_G the term containing $\exp(-k_F t)$ will disappear after some time and the equation will be reduced to

$$\ln \frac{(\alpha_t - \alpha_\infty)_{F+G}}{(\alpha_o - \alpha_\infty)_G} = -k_G t$$

This means that a plot of $\ln(\alpha_t - \alpha_\infty)$ *versus* time represents a curve which for large values of t becomes a straight line, with slope $-k_G$ and intercept $\ln(\alpha_o - \alpha_\infty)_G$. In Fig. 1 a graphical representation of this type based on experiments is shown. That this line represents the transformation of α-glucose to the equilibrium mixture of α and β-glucose is clear from the fact that the evaluated k_G is identical with the mutarotation constant obtained by dissolving

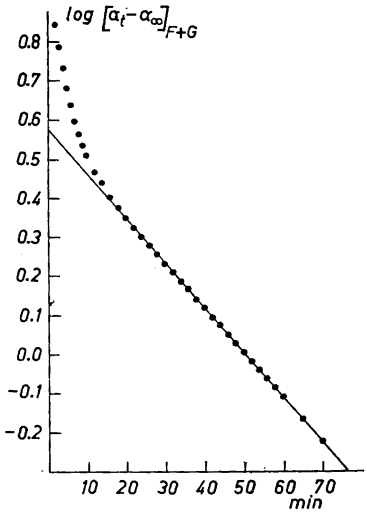

Fig. 1. The experimental values of log $(\alpha_t - \alpha_\infty)_{F+G}$ after rapid hydrolysis of sucrose. α_t represents the resulting optical rotation of the mutarotating glucose and fructose.

α-glucose in the same enzyme-buffer solution. Since we know $(\alpha_o - \alpha_\infty)_G$ and k_G we can calculate the value of $(\alpha_o - \alpha_\infty)_G \exp(-k_G t)$ at any t and subtract this value from the measured total value of $(\alpha_t - \alpha_\infty)_{F+G}$. In that way we obtain a set of values for t and $(\alpha_t - \alpha_\infty)_F$, representing a transformation of the β-fructofuranose to the equilibrium mixture. Our assumption was that this transformation exhibits first order rate behavior and if it holds, the reaction should follow the equation:

$$\ln \frac{(\alpha_t - \alpha_\infty)_F}{(\alpha_o - \alpha_\infty)_F} = -k_F t \qquad (V)$$

This means that by plotting $\ln(\alpha_t - \alpha_\infty)_F$ against time for the values obtained above, the curve should prove to be a straight line. Fig. 2 shows that a graphical

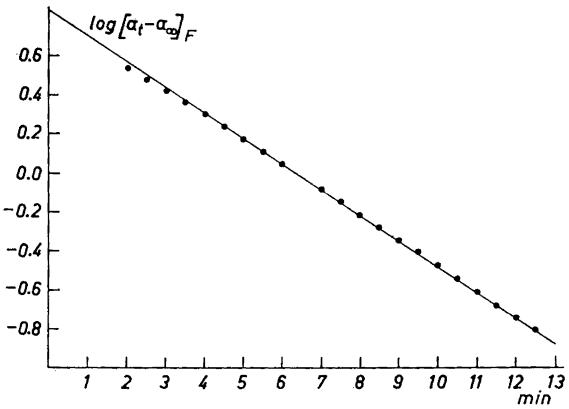

Fig. 2. The calculated values from the same experiment of log $(\alpha_t - \alpha_\infty)_F$ *versus* time. α_t represents the optical rotation of the mutarotating fructose.

representation of this type based on experiments is indeed a straight line, except for the first three minutes (we shall later return to this irregularity). This linear plot shows that the transformation of the liberated β-fructofuranose to its equilibrium mixture follows a first order equation and allows the velocity constant k_F for this reaction to be calculated. Further experiments showed that crystalline fructopyranose brought in solution under exactly the same conditions mutarotates with a velocity constant identical with that evaluated from Fig. 2.

We started with the assumption that the enzymatic splitting of the sucrose in our experiments was instantaneous. This of course is not possible even with the highly active β-fructofuranosidase we used [7]. With 2 mg of the enzyme we obtained a half life of 27 sec. This explains the irregularity of the curve in Fig. 2 mentioned before. The fact that it takes about 3 min. for the sucrose to be hydrolyzed does not affect the calculations up till now, but in the calculations below it cannot be neglected. The calculation of the optical rotation of β-fructofuranose is based on the fact that the straight line in Fig. 2 intersects the y-axis at $\ln(\alpha_o - \alpha_\infty)_F$. Since α_∞ can be measured with great accuracy, the value of α_o can be obtained. However, it is evidently not correct to extrapolate to zero time since at this time the concentration of β-fructofuranose is zero. The concentration of β-fructofuranose in the reaction mixture is determined by the two consecutive reactions I and III.

$$\underset{x}{S} \xrightarrow{k_S} \underset{y}{F_1} \xrightarrow{k_F} \underset{z}{F_2}$$

Assuming in this special case that the enzymatic splitting of sucrose is a first order reaction the following differential equations is found

$$-\frac{dx}{dt} = k_S x \; ; \; -\frac{dy}{dt} = -k_S x + k_F y \; ; \; \frac{dz}{dt} = k_F y$$

Integration of the first equation gives

$$-\ln x = k_S t + I$$

If the concentration of sucrose is a for $t = 0$

$$I = -\ln a$$

and $x = a \exp(-k_S t)$

Substituting this value of x in the second differential equation, we get

$$\frac{dy}{dt} = -k_F y + k_S a \exp(-k_S t)$$

From this first order linear differential equation, y is calculated.

$$y = \exp(-k_F t)\left(\frac{(k_S a \exp(k_F - k_S)t}{k_F - k_S} + P\right)$$

For $t = 0$, $y = 0$ and $P = -a\, k_S/(k_F - k_S)$. We thus obtain $y = -P \exp(-k_S t)$

$+ P \exp(-k_F t)$. Since k_S is far greater than k_F, the first term in this equation can be neglected for large t. We thus get $y = P \exp(-k_F t)$. The correct value of t to which the straight line in Fig. 2 has to be extrapolated is the time when the β-fructofuranose concentration is equal to the initial sucrose concentration. Setting $y = a$, in the equation gives $t = -\ln (a/P)/k_F$ and substituting this value of t in the first order eqn. (V) we obtain $\alpha = (a/P)(\alpha_o - \alpha_\infty) + \alpha_\infty$ in which α denotes the optical rotation of the untransformed β-fructofuranose. Inserting the value of P we get $\alpha = [(k_S - k_F)/k_S] (\alpha_o - \alpha_\infty) + \alpha_\infty$. The specific optical rotation $[\alpha]_{546}^{25}$ can be found from $[\alpha]_{546}^{25} = \alpha/LC$ where C is the concentration in g/cm³ and L the tube length in dm.

EXPERIMENTAL

Polarimetric measurements were performed at 25.0°C in a 10 cm polarimeter tube in a thermostated jacket. The polarimeter was a Zeiss »Lichtelektriches Polarimeter» which allowed readings of the optical rotation with an accuracy of 0.005°. The wavelength used was 546 mμ (the green Hg-line). The change of optical rotation was followed directly in the polarimeter with readings at intervals of 30 sec. The first value after the start of the reaction was usually at 90 sec.

All reaction mixtures consist of:

(1) Acetate buffer, pH 4.75, 0.005 M with respect of sodium acetate and 0.005 M with respect to acetic acid.

(2) One of the following sugars
Sucrose, 10 g/100 ml solution
Fructose, 5.263 g/100 ml solution
Glucose, 5.263 g/100 ml solution
(5.263 g of the monohexoses corresponds to 10 g of sucrose).

(3) Enzyme solution, 1 ml. (Added to the solutions of monohexoses only to assure identical conditions).

The enzyme solution contained 2 mg of β-fructofuranosidase per ml, after being adjusted to pH 4.75. This adjusting of pH was performed either with 1 M acetic acid (in the following refered to as enzyme solution *a*) or with 1 M acetate buffer pH 4.75 (refered to as *b*).

Two experiments with rapid hydrolysis of sucrose were carried out. The only difference between them was the use of enzyme solution *a* in one case, and *b* in the other. The difference in acetate ion concentration due to different adjusting of the pH in the enzyme solution is enough to change the rate constant for the fructose mutarotation. In a later publication we shall treat this problem. For the present it means that two lines of the type in Fig. 2 are obtained with different slopes and the same intercept, which means two different determinations of a_0 for the fructofuranose. (Figs. 1 and 2 refer to case *a*).

The determination of the mutarotation constants for α-glucose and β-fructopyranose was accomplished using the same conditions as in case *a*.

The value of k_S has been determined from experiments in which the 10 % sucrose solution was incubated for 3 min with different amounts of enzyme. With 2 mg, 99 % of the sucrose was hydrolysed after 3 min.

In all cases the slope and the intercept are calculated by means of the method of least squares.

RESULTS

Identity of rate constants

	Velocity constants derived from hydrolyzing sucrose. Enzyme solution *a*.	Velocity constants derived from mutarotating *a*-glucose and *β*-fructopyranose. Enzyme solution *a*.
k_G/ 2.303 min^{-1}	0.01087	0.01085
k_F/ 2.303 min^{-1}	0.0997	0.0943

Determination of optical rotation of the furanose form

	a.	*b*.
k_G/ 2.303 min^{-1}	0.01087	0.01144
k_F/ 2.303 min^{-1}	0.0997	0.1309
$\log(a_o - a_\infty)_G$	0.576	0.576
$\log(a_o - a_\infty)_F$	0.828	0.834
k_S min^{-1}		1.53

equilibrium mixture of 5.263 % glucose ($a_{\infty G}$): 3.29°
equilibrium mixture of 5.263 % fructose ($a_{\infty F}$): −5.52°

a (5.263 %) determined from the correction formula:

	a	*b*	Average
Glucopyranose:	6.99°	6.99°	6.99°
Fructofuranose:	+0.21°	−0.03°	0.09°

From this the specific optical rotation for the *β*-fructofuranose is determined; $[\alpha]_{546}^{25} = 1.7°$.

CONCLUSION

From the identity of rate constants it is proved that the transformation of the *β*-fructofuranose liberated from a glycosidic linkage consists of only one reaction, namely a transformation to an equilibrium mixture of furanose and pyranose. The composition of the equilibrium mixture at 25°C can now be calculated since the optical rotations of both forms and of the equilibrium mixture are known. The result, 31.56 % fructofuranose and 68.44 % pyranose, further permits the calculation of the specific optical rotation of the furanose form at any desired wavelength since the optical rotation of the pyranose and of the equilibrium mixture are always easily determined. In the case of sodium light (589 mμ) $[\alpha]_{589}^{25} = -4.58°$, to be compared with the discussed values from the literature.

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