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THE HYDROLYSIS OF SALICIN BY THE ENZYM EMULSIN.

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INTRODUCTION.

Salicin is a glucosid which occurs in many trees, particularly those of the willow family. In aqueous solution it is hydrolyzed by strong acids to glucose and salicyl alcohol according to the equation of Piria:[a] $C_{13}H_{18}O_7 + H_2O = C_6H_{12}O_6 + C_7H_8O_2$ (salicin + water = glucose + salicyl alcohol).

It has been found by A. A. Noyes and Hall[b] that the rate of this acid hydrolysis follows the law of unimolecular reactions. The same hydrolysis can also be accomplished by adding to the salicin solution a little of the enzym of almonds, called emulsin, but in this case it has been stated by Henri[c] and other investigators that the rate does not follow at all the unimolecular law. This statement that the enzymotic hydrolysis of salicin by emulsin does not follow the usual laws of chemical dynamics has passed unchallenged for many years and has been widely accepted as correct. In confutation of this view, it is to be said that the glucose which is liberated from salicin by the action of emulsin is doubtless β-glucose, because emulsin hydrolyzes only the β-glucosids, and β-glucose has a rotary power of 20°; but Henri in his work assumed that the glucose had its usual specific rotation of 52°. His polariscopic measurements of the rate of the enzymotic hydrolysis are accordingly incorrect, for he made no correction for the mutarotation of glucose. In the hydrolysis by acids as studied by A. A. Noyes and Hall this second reaction, the mutarotation of glucose, does not affect the estimation of the extent of the hydrolysis from the

[a] Liebig, Ann. Chem., 1845, *56:* 35.
[b] Zts. physikal. Chem., 1895, *18:* 240.
[c] Lois générales de l'action des diastases, p. 102.

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polariscopic readings, because the strong acid and the high temperature employed (95° C.) make the rate of the mutarotation instantaneous in comparison with the rate of the hydrolysis; but in the hydrolysis by emulsin the polariscopic readings do not give the real extent of the hydrolysis unless a considerable correction is made for the mutarotation of the freshly liberated glucose. The case is very similar to the hydrolysis of cane sugar by the enzym invertase, in which reaction the mutarotation of glucose, as has been shown by one of the writers,[a] plays an important part.

THE REAL AND APPARENT COURSES OF THE HYDROLYSIS OF SALICIN BY EMULSIN.

The emulsin consisted of two samples, one from a chemical manufacturer, who stated that it had been prepared from almonds, and a second, which we prepared by digesting powdered bitter almonds for a day in water, filtering through cloth, precipitating a casein-like substance with a little acetic acid, filtering, and precipitating the emulsin with alcohol as a flocculent white mass. The salicin was prepared by recrystallizing a kilogram of commercial salicin once from alcohol and then twice from water. Five grams of this dry product dissolved in 100 grams of water gave a solution of specific gravity 1.013 at 90° C., and of −14.91° rotation in a 50 cm tube; therefore the specific rotation of the pure salicin was −61.8°; Noyes and Hall[b] found −61.5° to −62.2° and Tiemann[c] found −62.6°. As it was expected that salicin on hydrolysis by emulsin would liberate β-glucose, which would slowly change partially to α-glucose, means were taken to stop the action of the emulsin at definite instants by adding a little sodium carbonate; the slightly alkaline solution was then read in the polariscope. Special experiments proved that the action of the emulsin is stopped instantly by the sodium carbonate and that the mutarotation of the glucose reaches completion almost instantly in the alkaline solution; the rotations of these alkaline solutions therefore show the real extent of the hydrolysis at the time the alkali was added. In the experiment reported in Table I, 500 cc of an emulsin solution were mixed at the time 0 with 20 grams of finely powdered salicin, the solution filtered after one minute's shaking, and the filtrate kept at 30° C. in a thermostat. A portion of it was used to fill a 50 cm jacketed observation tube, which was also kept at 30°. The readings of this portion, as shown in column 2 of Table I and curve I of figure 1, give the apparent course of the hydrolysis. It is this course which Henri measured.

[a] J. Amer. Chem. Soc., 1908, *30:* 1160–1166, 1564–1583; 1909, *31:* 655–664.

[b] Loc. cit.

[c] Ber., 1885, *18*:1600.

At intervals portions of the main solution were made slightly alkaline with sodium carbonate and their rotations read. These readings give the real course of the hydrolysis and are shown in column 3 of Table I and curve II of figure 1. The emulsin solution before the addition of the salicin had a rotation of −3.0° in a 50 cm length; as the addition of sodium carbonate to the emulsin in the absence of salicin did not change its rotation, the latter was corrected for by the subtraction of −3.0° from all the readings. As thus corrected, the readings were all referred to −62.0° as the specific rotation of salicin for sodium light, the factor by which they were multiplied being 5.17.

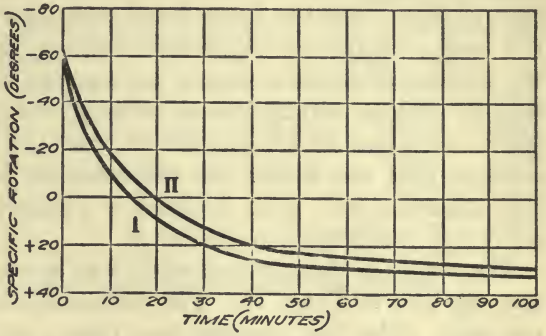

FIG. 1.—Real (I) and apparent (II) courses of the hydrolysis of salicin by emulsin.

TABLE I.—*Real and apparent courses of the hydrolysis of salicin by emulsin, temperature 30° C.*

Time (t).	Specific rotation.		Velocity-coefficient.[a]	
	Neutral solution (apparent course).	Alkaline solution (real course).	Apparent course.	Real course.
1.	2.	3.	4.	5.
Minutes.	*Degrees.*	*Degrees.*		
0	−62.0 (R_o)	−62.0		
10	−17.0		0.028	
20	.3	8.6	.024	0.030
30	13.7	23.7	.024	.035
40	19.4	25.6	.022	.029
50	22.5	27.6	.020	.026
75	27.6		.017	
95	29.2		.016	
∞	32.2 (R_∞)	32.2		
Average				.030

[a] Calculated by the usual formula, $k_1=\frac{1}{t}\log\frac{R_o-R_\infty}{R-R_\infty}$; the value of R_∞ is calculated from the molecular weights and specific rotations of salicin and glucose, as other experiments which have been made by the authors show that the hydrolysis of salicin by emulsin is a complete one.

The data of Table I show that the usual polariscopic method of following the hydrolysis by reading the rotation of the neutral or slightly acid solution gives wholly incorrect results. The true extent of the hydrolysis can only be found when means are taken to correct

for the mutarotation of the glucose. If the mutarotation is not corrected for, the apparent course of the hydrolysis is measured, and the data of column 4 show that this course does not follow the unimolecular law, since its velocity-coefficient decreases regularly during the reaction. On the other hand, the data of column 5 show that the course of the real hydrolysis follows the unimolecular law, for its velocity-coefficient shows no regular variation. These results largely explain why previous investigations, in particular those of Henri, have led to the conclusion that the hydrolysis of salicin by emulsin does not follow the unimolecular law, for Henri measured the apparent rate and obtained a regularly decreasing coefficient similar to that shown in column 4.

The real rate of hydrolysis at 0° was also measured, and the results are reported in Table II. The concentration of the salicin was 3.5 per cent. The first reading was taken one hour after mixing the salicin and emulsin because the temperature was somewhat above zero during this hour; this reading is accordingly lower than −62.0°, the specific rotation of salicin.

TABLE II.—*Real course of the hydrolysis of salicin by emulsin, temperature 0° C.*

Time (t).	Specific rotation of alkaline solution (real course).	Velocity-coefficient (k_1).
Hours.	*Degrees.*	
0	−37.5	.....
1	−13.8	0.073
2	−17.1	.075
3	−11.3	.068
5	− 2.4	.073
7.5	9.6	.065
9.5	15.6	.066
∞	32.2	.....
Average	.....	.070

This experiment shows a satisfactory constancy for the value of k_1, proving that the enzymotic hydrolysis follows the unimolecular law.

The real rate of hydrolysis was also measured at 30° C. in a third experiment, as shown in Table III. The salicin solution was of 5 per cent strength. In this experiment the action of the emulsin was stopped by quickly heating portions of the solution, a procedure which also brings the mutarotation to completion; it has the advantage over the addition of sodium carbonate that the red color which the latter forms with salicyl alcohol is not produced in such intensity and the polariscopic view is accordingly clearer.

TABLE III.—*Real course of the hydrolysis of salicin by emulsin, temperature 30° C.*

Time (t).	Rotation of alkaline solution (real course).	Velocity-coefficient (k_1).
Minutes.	*Degrees.*	
0	−62.0	0.00360
10	−54.5	.00330
20	−48.7	.00353
30	−41.6	.00339
35	−39.5	.00344
85	−15.8	.00350
145	+ 2.9	.....
∞	+32.2	.....
Average	.....	.00346

This experiment also shows a satisfactory constancy for k_1.

THE FORM OF GLUCOSE WHICH IS LIBERATED FROM SALICIN BY THE ACTION OF EMULSIN.

From the fact that the addition of alkali to the salicin solutions which are undergoing hydrolysis causes an increase of dextrorotation, it may be concluded that the glucose which is liberated from the salicin is less dextrorotatory than 52°, the rotation of stable or alkaline solutions of glucose. Only one form of glucose is known which has such lower rotation, namely, β-glucose, of specific rotation 20°. That this is indeed the form which is liberated from salicin may be accurately shown as follows:[a] Start with A molecules of salicin in unit volume of solution at constant temperature and let there be present at the time t, w molecules of fresh glucose and y molecules of stable glucose. The rates of formation at the time t are:

(1) For fresh glucose, $dw/dt = k_1 [A\text{-}w\text{-}y] - k_2 w$.

(2) For stable glucose, $dy/dt = k_2 w$.

In these expressions k_1 is the velocity-coefficient of the real rate of hydrolysis, and k_2 is the velocity-coefficient of the mutarotation of glucose. The solution of these equations under the conditions which obtain (that at the time zero, w, y, and dy/dt are all zero) is—

$$(3)\quad w = A\frac{k_1}{k_2 - k_1}\left[e^{-k_1 t} - e^{-k_2 t}\right]$$

$$y = A\left[1 + \frac{k_1}{k_2 - k_1}e^{-k_2 t} - \frac{k_2}{k_2 - k_1}e^{-k_1 t}\right]$$

[a] This mathematical treatment is closely analogous to that previously used in studying the inversion of cane sugar by invertase. See J. Amer. Chem. Soc., 1908, *30:* 1576.

In the experiment reported in Table I, the coefficient k_1 has the value 0.030, using decimal logarithms. At the temperature of the experiment (30°C) it was found in a previous investigation [a] that the velocity-coefficient of the mutarotation of glucose, k_2, had the value 0.017, and that the presence of salicin and emulsin in the solution had no effect on k_2. Substituting the values 0.030 and 0.017 for k_1 and k_2, respectively, in equation (3) the relative number of gram-molecules of fresh glucose (w/A) which were present in the solution of Table I at the different instants when the polariscopic readings were made, have been calculated and are given in column 3 of Table IV.

TABLE IV.—*Calculated specific rotation of fresh glucose from salicin.*

1.	2.	3.	4.
Time (t).	Change in rotation (D).	Fresh glucose (w/A)= W.	Calculated specific rotation of fresh glucose.
Minutes.	*Degrees.*	*Gram molecules.*	*Degrees.*
0	0.0	0.00	25
20	8.3	.48	15
30	10.0	.42	23
40	6.2	.34	20
50	5.1	.25	.....
∞	.0	.00	.....
Average	.....	.....	21

In column 2 are the changes of rotation caused by the addition of the sodium carbonate, obtained by subtracting in Table I column 3 from column 2. The last column of Table IV gives the specific rotation of the fresh glucose as calculated from the data of columns 2 and 3 by the formula which has been given in a former article,[b] specific rotation $= 52.5 + \frac{286D}{180W}$, where 52.5 is the specific rotation of stable glucose, 286 the molecular weight of salicin, 180 that of glucose, D the change in rotation of column 2, and W the per cent of fresh glucose in gram-molecules from column 3. The average value is 21°, and as the specific rotation of β-glucose has been found by Poux [c] to be 20°, and that of α-glucose 110°, the agreement proves that the form of glucose which is liberated from salicin by the enzymotic action of emulsin at 30° is β-glucose.

[a] J. Amer. Chem. Soc., 1908, *30:* 1577.
[b] J. Amer. Chem. Soc., 1908, *30:* 1581.
[c] Ann. chim. phys., 1903 [7], *30:* 422.

THE INFLUENCE OF ACIDS AND ALKALIS ON THE ACTIVITY OF EMULSIN.

The enzym emulsin is very sensitive to acids and alkalis. In Table V are recorded a series of measurements of the rate at which a constant quantity of emulsin hydrolyzed at 35° a 4 per cent solution containing different amounts of hydrochloric acid or sodium hydroxid. The results are shown also in figure 2.

TABLE V.—*Activity of emulsin toward salicin in acid and alkaline solutions.*

Experiment No.	Concentration of sodium hydroxid (gram-molecules per liter).	Activity of emulsin.[a]	Experiment No.	Concentration of hydrochloric acid (gram-molecules per liter).	Activity of emulsin.[a]
1	0.009	0	7	0.00009	222
2	.005	0	8	.00027	222
3	.0009	138	9	.0005	225
4	.0005	195	10	.0018	242
5	.00009	215	11	.005	255
6	(b)	222	12	.009	206
			13	.011	77
			14	.014	0
			15	.040	0

[a] The values of the activity are those of the velocity-coefficient of the hydrolysis (k_1), using decimal logarithms and minutes, multiplied by 100,000 to give whole numbers.
[b] Distilled water.

Emulsin is entirely inactive toward salicin except in the region just above neutrality. In this respect it resembles the enzym invertase, but the range of activity of emulsin does not extend into as strong acidity as does that of invertase, and extends farther into alkalinity. The emulsin solution which was used in the preceding experiments was dialyzed several days in order to remove as much as possible of any salts, acids, or alkalis that may have originally been in the infusion of almonds; the dialyzed solutions were neutral to litmus. Since the curve of activity (fig. 2) is not very steep at the point of neutrality, there is no need for making the emulsin solutions slightly acid in order to obtain steady and reproducible conditions for the hydrolysis. This is in strong contrast to the enzym invertase for which the curve is exceedingly steep near the point of neutrality, and the addition of acid to bring the rate into a region where the slope is less steep is quite important.

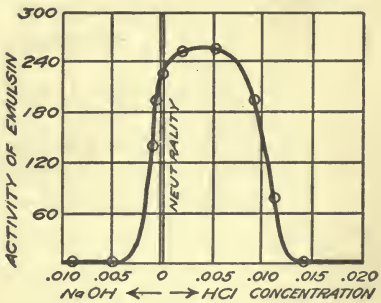

FIG. 2.—Influence of acidity and alkalinity on the activity of emulsin.

SUMMARY.

The conclusions of our investigation, which had for its purpose the development of an accurate polariscopic method for measuring the activity of the enzym emulsin, may be summarized as follows: The glucose which is produced from salicin by the action of emulsin is shown to have the rotation 15° to 25°, agreeing with the known rotation of β-glucose, 20°, and differing entirely from that of α-glucose, 110°. The glucose from salicin is therefore β-glucose. A secondary reaction, the mutarotation of glucose, affects the polariscopic readings of the salicin solutions during the hydrolysis by emulsin and is the chief source of error in the measurements of Henri, who found that the hydrolysis does not follow the unimolecular order. No such error is present in the measurements of the hydrolysis of salicin by acids, studied by A. A. Noyes and Hall, because the strong acid and the high temperature (95°) which were employed made the rate of mutarotation instantaneous in comparison with the rate of the hydrolysis; they found that the rate of the acid hydrolysis follows the unimolecular order. The authors have measured the real rate of the hydrolysis of salicin by emulsin at 0° and 30° by making the solution slightly alkaline before reading it in the polariscope, and this rate was found to follow the unimolecular order. Emulsin is active in only a small region of acidity and alkalinity near the neutral point, as shown in figure 2. It is intended to apply this accurate polariscopic method to a further study of the hydrolysis of salicin and other substances by emulsin, in order to learn the laws of the action of this enzym.

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