CXLV. THE ENZYMIC SYNTHESIS OF RAFFINOSE.

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IN a recent paper Vogel and Pictet [1928] described a synthesis of raffinose by heating sucrose with galactose in equimolecular quantities at 160–165° and 13–15 mm. The synthesis of raffinose from sucrose and galactose may also be effected at ordinary temperatures in the presence of an enzyme. My own investigations, made ten years ago but hitherto unpublished, gave several indications of the occurrence of such a synthesis.

According to Neuberg [1906] emulsin converts raffinose into sucrose and galactose. This reaction is very specific for raffinose and may be applied for the detection of this trisaccharide even in the presence of large amounts of sucrose [Neuberg and Marx, 1906]. Neuberg has shown that the hydrolysis of raffinose in aqueous solution is not complete, an equilibrium being set up, and this suggests the occurrence of a reverse reaction, the condensation of sucrose and galactose to form raffinose.

We used in our experiments Kahlbaum's preparations of galactose and raffinose, and Merck's emulsin and sucrose. Toluene was added to all the experimental solutions, and at the end of experiments they were quite sterile. The rate of hydrolysis was determined by the Lippich-Landolt polarimeter and by Bertrand's method. The samples were pipetted into the measuring flasks and weighed. The enzyme action was stopped by the addition of 1 cc. 2 % sodium carbonate solution and the solutions were diluted with water up to the mark. The rate of hydrolysis of raffinose by emulsin under the experimental conditions was very slow and even after 3 months the reaction had not reached an end. The differences in the initial values of rotation depend probably on the rotation of aqueous solutions of the emulsin, which in my experiments showed an $[\alpha]_D$ of 35.0°. This rotation is abolished in boiled solutions of emulsin.

Exp. 1. Raffinose 5.89 g., emulsin 0.1 g., water to 50 cc., toluene 0.4 cc. Room temperature.

Duration of experiment (days)	Rotation		Reducing sugar in 50 cc. (g.)
	Observed	$[\alpha]_D$	
0	+ 6.79°	+ 101.97°	0
4	6.75	101.29	0.526
8	6.68	100.19	0.602
18	6.66	99.85	0.775
41	6.53	97.94	1.075

The amount of reducing sugar in 50 cc. at the completion of hydrolysis would be 2.104 g. Consequently the hydrolysis after 40 days has reached only one-half of the possible value. In a control experiment with boiled enzyme hydrolysis is not observed.

In the following experiment the duration of hydrolysis was 80 days at 35° and the content of reducing sugar at the end amounted to 84 % of the possible theoretical value.

Exp. 2. Raffinose 11.78 g., emulsin 0.04 g., water to 25 cc., toluene 0.2 cc. Temperature 35°.

Duration of experiment (days)	Rotation		Reducing sugar in 50 cc. (g.)
	Observed	$[\alpha]_D$	
0	+ 6.81°	+ 102.20°	0
79	4.71	70.58	3.550

Our first experiments to show the existence of a reverse reaction gave negative results as is shown by Exp. 3.

Exp. 3. Sucrose 13.1308 g., galactose 6.9 g., emulsin 0.1 g., water to 100 cc., toluene 0.8 cc. Temperature 35°.

Duration of experiment (days)	Rotation		Galactose in one sample (mg.)
	Observed	$[\alpha]_D$	
0	+ 4.64°	+ 69.68°	55.2
7	4.60	69.64	55.4
15	4.67	70.04	55.6
46	4.69	70.41	54.6
60	4.66	69.92	55.2

These negative results which were constantly obtained in aqueous solutions obliged me to seek the solution of the problem in the use of a non-aqueous medium. This ought to satisfy three conditions: (1) to dissolve the galactose and sucrose readily, (2) to be indifferent towards these sugars, and (3) not to inactivate the emulsin. According to Bourquelot [1913] these conditions are satisfied by acetone in 80 % solution in water. Emulsin is insoluble in acetone and the experimental solutions must be shaken at intervals. The solubility of galactose in acetone is relatively small (about 17 %) and in our first experiments we prepared saturated solutions of this sugar in acetone and after filtration added the sucrose. The experiments were made only in one direction, from galactose and sucrose towards raffinose. The results obtained indicate the possible presence of a synthetic reaction.

Exp. 4. Saturated solution of galactose in 80 % acetone 50 cc., sucrose 2.94 g., emulsin 0.23 g. Temperature 35°.

Days from beginning	α_D
0	+ 8.64°
78	10.58

Exp. 5. Sucrose 3 g., galactose 1.5 g., 80 % acetone 100 cc., emulsin 0.2 g. The solution was boiled. Temperature 35°.

Days from beginning	α_D
0	+ 5.70°
47	5.57

Exp. 6. Saturated solution of galactose in acetone 100 cc., sucrose 5.87 g., emulsin 0.2 g., 80 % acetone to 200 cc. Temperature 35°.

Days from beginning	α_D
0	+ 4.68°
108	5.27

Exp. 7. Galactose 3 g., sucrose 6 g., emulsin 0.5 g., 80 % acetone to 200 cc. Temperature 35°.

Days from beginning	α_D
0	+ 5.53°
100	5.97

In order to show that the increase of rotation is due to the synthetic action of emulsin, 50 cc. of solution from *Exp. 6* were evaporated *in vacuo*. The residue was dissolved in water and 0.2 g. of emulsin added. After 3 months the rotation changed from + 5.57° to + 5.12° and the copper-reducing power increased from 0.2975 g. to 0.3885 g. (calculated for galactose).

For the preparation of the synthetic product in more or less pure form we made the following experiment. Galactose and sucrose were dissolved in equimolecular proportions (17.9712 g. of galactose and 34.1765 g. of sucrose) in 1000 cc. of 80 % acetone. 1 g. of emulsin was added and the mixture was placed in a thermostat at 35° for 5 months. The rotation changed from + 5.40° to + 5.68°. The acetone was distilled off *in vacuo*, and the solution was concentrated by evaporation to the consistency of a dense syrup and extracted by boiling 96 % ethyl alcohol. After a few days large crystals of sucrose and crystalline aggregates of galactose separated from the alcoholic solution. The mother liquor was evaporated and the residue again extracted by alcohol. After a new crystallisation of sucrose and galactose the mother liquor was decanted and after standing for some time deposited small crystals. These crystals were washed by alcohol and dried. 0.4117 g. was dissolved in 25 cc. of water and after 24 hours gave, in a 2 dm. tube, a rotation $\alpha_D = + 3.15^\circ$, corresponding to the specific rotation $[\alpha]_D = + 95.66^\circ$. (For raffinose $[\alpha]_D = + 104^\circ$, for galactose + 81° and for sucrose + 66.5°.) To this solution emulsin was added. The rotation changed to + 2.81° and after 10 days decreased to $\alpha_D = + 2.34$. These results give us the right to conclude that the action of emulsin on a solution of galactose and sucrose in 80 % acetone produces a compound with the properties of raffinose.

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