The following table gives the properties, yields and analyses of the pyrazolones which were prepared.

TETRAHYDRO-2-PHENYL-PYRAZOLOPYRIDINE-3(3a)-ONES (I AND II)

Compound Type	R is	Form isolated	Formula	M. p., °C.	Yield, %	Analyses. N[a], % Calcd.	Found
I	Methyl	HCl	$C_{13}H_{16}N_3OCl$	224–225	70	15.82	15.76
II		HCl	$C_{13}H_{16}N_3OCl$	191–193	30	15.82	15.71
I	Ethyl	HCl	$C_{14}H_{18}N_3OCl$	187–188	68	15.03	14.91
I	*n*-Propyl	HCl	$C_{15}H_{20}N_3OCl$	191–192	65	14.31	14.51
I	*n*-Butyl	Base	$C_{16}H_{21}N_3O$	117–118	40	15.50	14.60[b]
I	Isoamyl	Base	$C_{17}H_{23}N_3O$	125–126	60	14.74	14.61

[a] Semi-micro Dumas. [b] No explanation can be advanced as to why the analyses for this particular compound persisted in being so far below the calculated value.

Attempted Preparation of 4,5,6,7-Tetrahydro-2-phenyl-1,5-dimethyl-2,1,5-pyrazolopyridin-3(3a)-one.—(a) Five grams of the pyrazolone (I, R is methyl) hydrochloride was dissolved in 10 g. of 20% sodium hydroxide. This alkaline solution was cooled in ice and 3 g. of dimethyl sulfate added slowly with stirring.[4] The solution was heated on the steam-bath for ten minutes to destroy the unreacted dimethyl sulfate. On cooling no precipitate of the alkali-insoluble 1-methyl derivative was apparent. The solution was then saturated with sodium hydroxide whereupon an oil precipitated which on account of its insolubility in ether and benzene and its solubility in water and chloroform was judged to be a quaternary compound.

(b) Five grams of the pyrazolone (I, R is methyl) hydrochloride and 20 cc. of methyl alcohol containing 35% hydrogen chloride[6] were heated in a sealed tube for two hours at 140°. The crystalline product obtained from the reaction mixture was the unchanged pyrazolone hydrochloride.

(c) An intimate mixture of 1 g. of 1-methyl-3-carbethoxy-4-piperidone hydrochloride and 0.72 g. of *sym*-methylphenylhydrazine hydrochloride was heated under a reflux condenser as in the reaction described above with phenylhydrazine hydrochloride. The reaction mixture was dissolved in water and the solution made alkaline with 10 cc. of 25% sodium hydroxide. An oil separated which was completely soluble in ether. No crystalline product could be isolated from this ethereal solution. Dry hydrogen chloride gave a gummy precipitate from this ethereal solution and this precipitate, likewise, could not be caused to crystallize.

Summary

A number of pyrazolones have been prepared from the isomeric and homologous 1-alkylcarbethoxypiperidones by the condensation of the piperidone hydrochloride with phenylhydrazine hydrochloride. It has not been possible to methylate these pyrazolones to analogs of antipyrine.

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The Analysis of Gamma-Methylfructoside Mixtures by Means of Invertase. I[1]

BY C. B. PURVES AND C. S. HUDSON

Although the sirupy mixture of non-reducing fructose derivatives, formed from fructose by methyl alcoholic hydrogen chloride, was discovered by Fischer[2] as early as 1895, the literature records no successful attempt to separate its constituents in crystalline form. This handicap did not prevent Menzies[3] and the earlier authors, whose work he reviewed, from showing that the elementary analysis of the mixture approximated to that of a methylfructoside; that the greater portion consisted of readily hydrolyzable fructose derivatives of a gamma type and that the latter, on methylation and subsequent hydrolysis, yielded a liquid tetramethylfructose similar to the specimens derived from sucrose and from inulin. More recently Schlubach and Rauchalles[4] followed up an old observation of Fischer[2] and hydrolyzed a portion of the liquid fructoside sirup back to fructose by means of invertase. After noting the consequent change in the copper reduction and optical rotation of the solution, the authors were led through a mathematical error to assign to the hydrolyzable constituent a specific rotation of $[\alpha]_D$ −17° instead of the value correct for their data, −51.5° in water.[5] In company with the above authors, the present paper occasionally implies that the enzyme exerts its specific effect upon a true methylfructoside of molecular weight 194. Although this assumption was justified in part by the analyses of the liquid methylfructoside mixture, fructose mono- and

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

(2) Fischer, *Ber.*, **28**, 1160 (1895).

(3) Menzies, *J. Chem. Soc.*, **121**, 2238 (1922).

(4) Schlubach and Rauchalles, *Ber.*, **58**, 1842 (1925).

(5) In Schlubach and Rauchalles' calculation the 33% of the gamma-methylfructoside mixture which was hydrolyzed by invertase and denoted by β contributed an optical rotation of $[\alpha]_D$ −17° *to the whole*. The specific rotation of the portion β *by itself* was therefore −17 × 100/33 or −51.5°. For the same reason the specific rotation of the remaining non-hydrolyzed 67% of the original was not +36.36° but 36.36 × 100/67 or $[\alpha]_D^{20}$ +54.3°.

dimethyl acetal or even a difructoside might well be included in it without seriously altering the average carbon, hydrogen and methoxyl content of the whole.

In the course of a study on invertase, it became important to confirm the specific rotation of the non-reducing fructose derivative unstable to the enzyme and the work of Schlubach and Rauchalles has been repeated in greater detail. After condensing the fructose with the methyl alcohol containing hydrogen chloride, the acid was neutralized and water was substituted for alcohol as the solvent. On the addition of a sufficiently powerful invertase solution the initial slight dextrorotation of the fructoside mixture increased rapidly to a maximum and then underwent a slower decrease to a value which remained constant for at least two days. Schlubach and Rauchalles predicted the form of this optical curve (Fig. 1) on the assumption that invertase hydrolyzed a gamma-methylfructoside of the beta configuration to a more dextrorotatory β-gamma fructose. The rotation of the latter then changed to the strongly levorotatory value of ordinary fructose in water.

In order to follow the actual progress of the reaction independently of the optical secondary changes, the increase in fructose was estimated by a copper reduction method and the data were plotted in Fig. 1 as minutes against the percentage of the total enzymotic hydrolysis. A similar hydrolysis curve was also made for an inversion of sucrose which was carried out, for purposes of comparison, with conditions as nearly as possible identical. The two hydrolysis curves were similar in form and were used to calculate the velocity constants K_s and K_f of the sucrose and fructoside, respectively, when the hydrolyses were at comparable degrees of completion. Table I summarizes the computations made on the assumption that both reactions were unimolecular in type.

TABLE I

HYDROLYSIS OF GAMMA METHYLFRUCTOSIDE AND OF SUCROSE BY INVERTASE

Hydrolysis, %		10	20	30	40
10^3K_s		423	461	489	512
10^3K_f		29.5	34.6	35.2	37.0
Ratio K_s/K_f		14.3	13.3	13.9	13.8
Hydrolysis, %	50	60	70	80	90
10^3K_s	540	546	555	578	594
10^3K_f	38.6	40.2	43.6	46.3	43.7
Ratio K_s/K_f	14.0	13.6	12.7	12.5	13.6

Although the material rise in both series of velocity constants as the hydrolyses progressed indicated that neither was strictly unimolecular, the ratio K_s/K_f = 13.5 was constant within the experimental error throughout the range of 10 to 90% hydrolysis for which the measurements were reliable. This parallelism between sucrose and the fructose derivative in their behavior toward yeast invertase lent support to the view that the mechanism of the inversion of sucrose by this enzyme was confined in its essentials to the fructose portion of the molecule. The other inference, that under comparable conditions sucrose

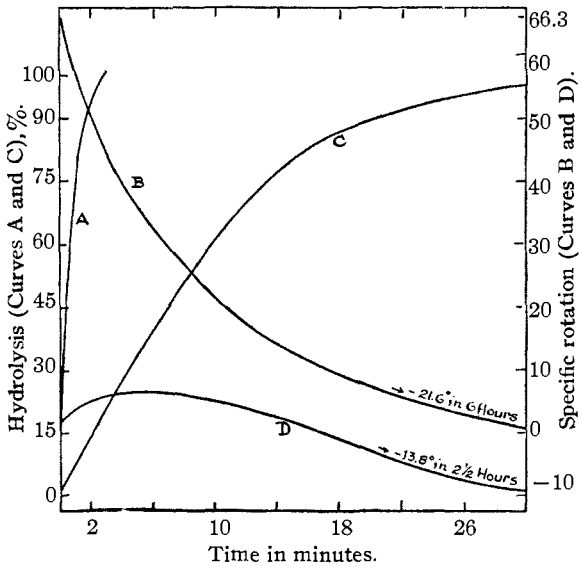

Fig. 1.—Hydrolysis of sucrose and gamma-methylfructoside by invertase: Curve A, sucrose, copper reduction; Curve B, sucrose, specific rotation; Curve C, gamma-methylfructoside, copper reduction; Curve D, gamma-methylfructoside, specific rotation.

was hydrolyzed by invertase about 13.5 times as rapidly as the gamma-methylfructoside, was only true provided this ratio was not disturbed by any possible effect on the activity of the enzyme of the glucose liberated in the first case or of the unchanged, non-reducing fructose derivatives present throughout in the second.

When the action of invertase upon the gamma-methylfructoside had reached completion, the changes in the optical rotation and fructose content of the solution made it possible to calculate the specific rotations and the absolute concentrations of the portions stable and unstable to the enzyme. In the case quoted experimentally, the stable fraction had a specific rotation of $[\alpha]_D^{20}$ +50.9° in water, and a concentration, as fructose,

of 2.62%. The indirect nature of these determinations made it desirable to check their accuracy by an independent method. Accordingly, all fructose was removed by a fermentation with yeast and the resulting non-reducing solution was found to contain 2.64% of gamma fructosides with a specific rotation of $[\alpha]_D^{20}$ +50.7°. The close agreement of these experimental figures with those calculated made it clear that only one of the constituents of gamma-methylfructoside sirup was affected by any of the enzymes of yeast and that it was invertase which hydrolyzed the constituent in question. Another conclusion of obvious experimental importance was that the remaining gamma-fructose derivatives were stable for three days in dilute aqueous solution at the P_H of 4.5 and the temperature of 38° used in the fermentation.

It was apparent from the above that one non-reducing component of gamma-methylfructoside might be estimated with reasonable accuracy by means of invertase. The copper reduction at any stage gave a measure of the fructose content of the solutions and the increase in reducing fructose, caused by acid hydrolysis with gamma conditions, could be attributed to the total non-reducing derivatives of a gamma nature which were present. Any difference between the latter and the total amount of fructoside sirup was due to methylfructosides much more stable toward acids and presumably of the normal ring type. By working on these lines, a system of analysis was developed which was used to determine the percentages of fructose and of the gamma fructosides, stable and unstable to invertase, present in many specimens of the gamma-methylfructoside mixture. It will be remembered from the work of Menzies[3] that in the condensation of the ketose with acid methyl alcohol the initial large levorotation of fructose very rapidly diminished to a value which was frequently slightly dextrorotatory. The first eight analyses, briefly summarized at the end of the experimental portion, refer to products obtained by arresting the reaction near the point of maximum dextrorotation. Menzies has already shown that the fraction of the fructose left uncombined at this point increased with the concentration of the water formed in the condensation, or, in other words, with the concentration of fructose in the original methyl alcoholic solution. The data fully supported this view and in analysis 7 the effect of a small amount of extraneous moisture was marked. A decrease in the normality of the hydrogen chloride used also tended to make the condensation less complete.

In contrast to the variable fructose content of the gamma-methylfructoside sirup, the composition of the non-reducing remainder was remarkably constant. In all cases about 45% was hydrolyzed by invertase and had a specific rotation, in water, of $[\alpha]_D^{20}$ −52 ± 2°. The rotation of the other 55%, stable to the enzyme and not fermented by yeast, was about $[\alpha]_D^{20}$ +50° in water. These values were significantly altered neither by varying the concentration of the fructose in the methyl alcohol from 2 to 10%, nor that of the hydrogen chloride from 0.0263 to 0.54 normal, nor by carrying out the condensation at 60° (analysis 3) instead of at 20°.

When the action of methyl alcoholic hydrogen chloride on fructose was prolonged for hours, the rotation of the solution again became strongly levorotatory and nearly constant. This change was attended by a slight diminution in the reduction of the solution and invertase hydrolyzed 10–20% instead of 45% of the non-reducing product. The specific rotation of this fraction, however, remained unchanged at $[\alpha]_D^{20}$ −52° (analyses 8–11) and the constancy of the rotation while the proportion present varied furnished evidence that invertase acted not upon a mixture but upon a definite chemical individual. The decrease in the percentage amount of this gamma sugar derivative, together with the decrease from $[\alpha]_D^{20}$ +50° to $[\alpha]_D^{20}$ −45° in the specific rotation of the portion unaffected by the enzyme, was accompanied by the accumulation in the acid methyl alcoholic solution of levorotatory fructoside of the normal ring type.

Experimental

Analytical.—Throughout the work all optical measurements were made on a high precision saccharimeter capable of being read to ±0.03°V. and with a conversion factor of 1°V. = 0.3462° circular. A 1.000% solution of pure fructose, $[\alpha]_D^{20}$ −92.16° in water, when read in a 4-dm. tube on this instrument had a levorotation at 20° of −10.65° in water, and of −10.85° in 0.62 normal saline. These standard rotations were used in the calculations.

The quantitative hydrolysis to fructose of non-reducing gamma-fructose derivatives was carried out by an adaptation of Herzfeld's[6] method for the inversion of sucrose. To 14 ml. of the solution in a 25-ml. flask, 1 ml. of concentrated hydrochloric acid was added and the flask and its contents were warmed for exactly ten minutes in a bath at

(6) Herzfeld, *Z. Ver. Zuckerind.*, **38**, 699 (1888); Browne, "Handbook of Sugar Analysis," p. 266.

69°. After cooling, the volume was made up to 25 ml. with normal caustic soda. An aliquot of this, suitably diluted and neutralized to phenol red with $N/10$ caustic soda, was estimated for fructose with the Shaffer–Hartmann reagents.[7] Tests indicated that under these conditions the hydrolysis of β-methylglucoside was less than 1%. In using the Shaffer–Hartmann method the potassium iodide was omitted from the alkaline copper solution but was added (1 ml. of a 5% solution) just before the final acidification and titration. Solutions containing known amounts of the pure sugar were used to calibrate the titrations in terms of milligrams of fructose with an accuracy well within ±2%. The estimations were frequently duplicated with consistently concordant results and the standardization against fructose was repeated occasionally.

The solution of invertase used in the selective hydrolysis was quite free from maltase and emulsin and 1 ml. added at 20° to 20 ml. of 10% sucrose solution at P_H 4.5 hydrolyzed 75.25% of the sugar in seventeen minutes. Although colorless and non-reducing, the enzyme solution had a dextrorotation of +0.17°V. when observed in a 4-dm. tube. An appropriate correction for this activity has been made when necessary in the optical measurements. The latter, together with the hydrolyses and all associated determinations, were made in a room kept at 20 ± 0.2°.

Preparation of Gamma-methylfructoside.—To 5 g. of analytically pure fructose dissolved in 100 ml. of pure, synthetic, acetone-free methyl alcohol contained in a distilling flask, was added, at 20°, 20 ml. of methyl alcohol made 3.0 normal with respect to dry hydrogen chloride immediately before use. After four minutes, when it was known that the dextrorotation of the solution was near the maximum, the liquid was made just alkaline to litmus. Normal aqueous caustic soda, 62 ml., was used in preference to silver carbonate in this instance, because the sodium chloride formed had little or no effect on the subsequent determinations or on the activity of invertase, which might have been partially inhibited by a trace of colloidal silver. After removing the whole of the alcohol by evaporation under diminished pressure, the reaction of the solution was changed from P_H 8 to 4.5 with dilute acetic acid and the volume was made up to 50 ml. with distilled water. The clear colorless 1.24 N sodium chloride solution corresponded in concentration to 10.00% fructose and had a dextrorotation in a 2-dm. tube of +0.734°V. or a specific rotation, as fructose, of $[\alpha]_D^{20}$ +1.3°. An initial reduction of 0.278% increased to 9.80% by a Herzfeld hydrolysis, which indicated the presence in the gamma-methylfructoside of fructose 2.8%, normal glycoside of the stable ring type 2% and non-reducing derivatives of gamma-fructose 95.2%.

Partial Hydrolysis of Gamma-methylfructoside with Invertase. Copper Reductions.—At 20°, zero time and at P_H 4.5, 10 ml. of the above fructoside solution was quickly mixed with an equal volume of the enzyme solution. Thereafter, at accurately known intervals, the hydrolysis was instantly and completely arrested in 1-ml. samples by discharge into 10 times the volume of 0.03 N sodium carbonate. These mixtures were permanently alkaline to phenol red and after suitable dilution were estimated for fructose in the usual way. The fructose content did not increase on standing. From the initial reduction, R_0, taken as 0.278/2 or as 0.139, and the final reduction, R_∞, estimated as 2.25; 2.28 or 2.265%, the per cent. hydrolysis Hy was calculated from the reduction, R, at intermediate times as $100(R - R_0)/(R_\infty - R_0)$. The velocity constant K_f of the hydrolysis was given by the unimolecular relationship $K_f = 1/T \log (100/(100 - \mathrm{Hy}))$.

(7) Shaffer and Hartmann, *J. Biol. Chem.*, **45**, 349 (1923).

TABLE II

PARTIAL HYDROLYSIS OF GAMMA-METHYLFRUCTOSIDE BY INVERTASE. COPPER REDUCING POWER

Min.	Hy, %	10^3K_f	Min.	Hy. %	10^3K_f
0	0	..	8.0	51.1	38.8
0.5	2.49	21.9	9.0	56.0	39.6
1.0	6.00	26.9	12.0	70.3	43.9
1.5	9.97	30.4	15.0	79.6	46.0
2.0	13.6	31.7	16.0	83.2	48.4
2.5	17.2	32.8	20.0	89.0	47.9
3.0	21.6	35.2	24	94.2	51.5
3.5	23.3	(32.9)	28	96.6	52.8
4.0	27.2	34.5	32	96.3	(44.6)
4.5	30.5	35.1	36	97.7	45.4
5.0	33.9	36.0	40	96.7	37.0
6.1	40.7	37.2	62	98.4	29.1
7.0	44.8	36.9	48 hrs.	100	..

The cessation of the partial hydrolysis was due neither to inactivation nor to a change in the reaction of the mixture, because at the end the solution rapidly inverted sucrose and had retained its original P_H of 4.5.

Partial Hydrolysis of Gamma-methylfructoside by Invertase. Optical Rotations.—The same fructoside solution was mixed with an equal volume of the invertase precisely as described above and the readings were made in a 4-dm. tube on the saccharimeter. An initial rotation of +0.734°V. was assumed and the specific rotations were calculated on the basis of a 5% fructose solution.

TABLE III

PARTIAL HYDROLYSIS OF GAMMA-METHYLFRUCTOSIDE BY INVERTASE. OPTICAL ROTATIONS

Min.	V° (obs.)	$[\alpha]_D^{20}$	Min.	V° (obs.)	$[\alpha]_D^{20}$
0	(+0.734)	+1.3	18.3	−0.83	− 1.5
1	2.13	3.7	19.7	−1.51	− 2.6
1.5	2.33	4.05	20.8	−2.07	− 3.6
2	2.81	5.05	22.1	−2.70	− 4.7
2.5	3.04	5.3	23.7	−3.58	− 5.9
3.25	3.27	5.7	25.5	−4.08	− 7.1
4	3.52	6.1	27	−4.70	− 8.2
4.75	3.62	6.3	29	−5.28	− 9.2
5.25	3.71	6.4	29.8	−5.45	− 9.5
6	3.71	6.4	32.5	−6.08	−10.6
7	3.62	6.3	35.3	−6.53	−11.4
8.2	3.46	6.0	36.7	−6.75	−11.7
9.25	3.22	5.6	37.8	−6.88	−12.0
10.5	2.77	4.8	40.3	−7.08	−12.3
11.2	2.59	4.5	41.2	−7.16	−12.4
12.5	2.10	3.7	135	−7.88	−13.7
13.75	1.49	2.5	400	−7.93	−13.8
14.5	1.12	1.95	Hours		
16.1	0.32	0.6	22	−7.87	−13.7
17.2	− .23	− .4	40	−7.92	−13.8

At the end the solution had a P_H of 4.4, contained 2.265% of fructose and rapidly inverted added sucrose. The final optical rotation was taken to be −7.89°V. in a 4-dm. tube.

Mathematical.—Let R_1 and R_2 represent the rotations contributed to the original gamma-methylfructoside solution by the non-reducing constituents respectively stable and unstable to invertase, and C and C_2 their percentage concentrations as fructose. The fructose present as such in the solution was 0.139% initially and 2.265% finally and C_2 was equal to the difference of 2.13%. In addition, as a 1% solution of the ketose had a levorotation of −10.85°V. in the experimental conditions (p. 704, line 7 of experimental) the rotation due to fructose in the two cases was (−10.85) × 0.139 or −1.51°V. and (−10.85) × 2.265 or −23.58°V. Prior to the partial hydrolysis

$$R_1 + R_2 - 1.51° = V_1$$

and after the action of the enzyme was complete

$$R_1 - 23.58° = V_2$$

where V_1 and V_2 were the rotations, +0.73°V. and −7.89°V., actually observed in the 4-dm. tube. These equations gave a value for R_1 of +16.69°V. and of −14.49°V. for R_2. The specific rotation of the glycoside hydrolyzed by invertase was then found from R_2 and C_2 to be $[\alpha]_D^{20}$ −58.9° (circular) as fructose (mol. wt. 180), $[\alpha]_D^{20}$ −50.0° as a fructose monomethyl acetal (mol. wt. 212) and $[\alpha]_D^{20}$ −54.6 as a true methyl fructoside (mol. wt. 194). In determining the concentration C_1 of the constituents unaffected by the enzyme, it was convenient to neglect the small amount of normal fructosides present which were more resistant to aqueous acid and to accept as the total potential fructose content of the solution the 4.90% revealed by the Herzfeld gamma hydrolysis. C_1 was given by the difference between this and the final fructose concentration of 2.265% or was 2.635%. The corresponding specific rotation in 0.62 N sodium chloride was (C_1, R_1), $[\alpha]_D^{20}$ +54.7° as fructose or $[\alpha]_D^{20}$ +50.9° as methylfructoside.

A Fermentation with Yeast.—Monobasic sodium phosphate, 10 mg., ammonium acetate, 10 mg., and starch-free baker's yeast, 250 mg., were added to 20 ml. of the above solution containing invertase before it was kept at 38° for forty-eight hours. In a control experiment, 20 ml. of a 2.28% fructose solution in 0.62 N sodium chloride was fermented in the same way. The losses in volume during the incubations were negligible and after freeing the solutions from yeast cells two water-clear non-reducing filtrates were obtained. That containing the gamma-methylfructosides had a rotation, in a 2-dm. tube, of +8.33°V. and a reduction of 2.64% after a Herzfeld hydrolysis, while the corresponding data for the yeast control were −0.01°V. and a zero reduction. The gamma-fructosides therefore had a concentration of 2.64% as fructose, a rotation of +8.34°V. in a 2-dm. tube and a specific rotation, as methylfructoside, of $[\alpha]_D^{20}$ +50.7°; in excellent agreement with the foregoing calculations.

Inversion of Sucrose by Invertase. Copper Reductions.—A solution was made up in 1.24 N sodium chloride, buffered to P_H 4.5 with $N/200$ sodium acetate and acetic acid, to contain 0.228% of fructose and 8.470% of sucrose (0.248 molar). The molar concentration of sucrose was twice that of the fructose liberated by invertase in the above gamma-methylfructoside solution, and the optical rotation of the mixture, in a 2-dm. tube, was 31.19°V. After mixing equal volumes of this solution with the invertase at 20°, the reduction as fructose was taken to be 0.114% initially, and was determined to be 4.51% when the inversion was complete.

The fructose content of the solution, the percentage hydrolysis and the unimolecular velocity constants, with one minute as unit time, were obtained at intermediate stages by the methods already described.

TABLE IV

INVERSION OF SUCROSE BY INVERTASE. COPPER REDUCTIONS

Sec.	Hy. %	10^3K	Sec.	Hy, %	10^3K
0	0	..	96	88.7	592
18	28.1	477	111	90.8	560
34	50.6	540	128	96.9	[686]
50.5	66.0	557	143	96.6	[616]
67	76.9	570	185	100	..

Final P_H checked at 4.5.

Inversion of Sucrose by Invertase. Optical Rotations.—The mixture containing equal volumes of the sucrose and enzyme solutions was observed at 20° and P_H 4.5 in a 4-dm. tube. An initial rotation of +31.19°V. was assumed and the specific rotations were calculated for a 4.235% sucrose solution after allowance was made for the rotation contributed by the fructose originally present; (−10.85) × 0.114 or −1.24°V. in a 4-dm. tube.

TABLE V

INVERSION OF SUCROSE BY INVERTASE. OPTICAL ROTATIONS

Min.	V.°	$[\alpha]_D^{20}$	Min.	V.°	$[\alpha]_D^{20}$
0	[+31.19]	66.3	20	+ 2.31	+ 7.3
1.3	25.89	55.4	24	+ 0.81	+ 4.2
1.8	23.31	50.2	25	+ 0.53	+ 3.6
2.8	21.11	45.7	30	− 0.81	+ 0.9
3.3	19.95	43.3	32	− 1.27	− 0.6
4.1	18.36	40.1	35	− 1.79	− 1.1
5	16.63	36.5	40	− 2.64	− 2.9
6.2	14.68	32.5	50	− 3.94	− 5.5
7	13.41	29.9	56.3	− 4.64	− 7.0
8	12.01	27.1	60.3	− 4.99	− 7.7
9.1	10.46	23.9	62	− 5.19	− 8.1
10	9.33	21.6	63	− 5.29	− 8.3
11	8.43	19.8	64.3	− 5.38	− 8.5
12	7.41	17.7	80.5	− 6.69	−10.7
13	6.46	15.7	190	−10.57	−19.1
15.1	4.96	12.7	370	−11.80	−21.6
17	3.86	10.4	29 hr.	−11.81	−21.6

The reaction of the solution after the inversion was P_H 4.4 and the final specific rotation of $[\alpha]_D^{20}$ −20.5 (corr. for change in mol. wt.) was in agreement with that of invert sugar.

Summary of Partial Analyses of Gamma-methylfructoside Sirup.—The second, third and fourth columns in the following table show the concentration of fructose and of hydrogen chloride used in each condensation and the time which elapsed before the product was isolated from the

methyl alcoholic solution. All the condensations with the exception of number 3 were carried out at 20°. The fifth column indicates the percentage of fructose left uncombined in the product as isolated while the remainder of the data in columns six to nine are corrected for this fructose content. In analyses 1–8 the total potential fructose concentration of the solutions was assumed to be that determined after a Herzfeld hydrolysis while in analyses 9–11 the data were based on the actual amount of fructose condensed with the acid methyl alcohol. The specific rotations of the non-reducing derivatives, both stable and unstable to invertase, were calculated for true fructosides of molecular weight 194.

TABLE VI

PARTIAL ANALYSES OF THE GAMMA-METHYLFRUCTOSIDE MIXTURES

	Condition of preparation			Analyses of product				
					Unstable		Stable	
Anal.	Fructose, %	HCl N	Min.	Fructose, %	%	$[\alpha]_D^{20}$	%	$[\alpha]_D^{20}$
1	0.474	0.0263	45	0	..	....	..	....
2	2.0	.0263	35	1.3	46	....	54	....
3[a]	2.0	.0263	3	1.6	44	−50.3	56	+46.5
4	10.0	.0263	130	10.0	46	−53.7	54	+51.3
5[b]	5.0	.50	4	2.8	45	−54.6	55[b]	+50.9
6	7.0	.50	4	3.6	45.6	−52.0	54.4	+48.0
7[c]	6.15	.50	5	11.5	40.3	−50.0	59.7[c]	+13.0
8[d]	8.0	.54	4	6.2	45	....	55	+48
9	10.0	.41	150	4.4	17.4	−50.7	82.6	−45.3
10	1.0	.50	1140	1.8	20.5	....	79.5	−46.4
11	7.5	.5	138 hours	1.4	10.0	−52.0	90.0	−36.3
12	Fractionated from EtOAc			0	53.3	−53.7°	46.7	+44°

[a] Condensation at 60°. [b] Previously described in detail. Contains 2% of normal fructosides. [c] Extraneous moisture present. 59.8% of the non-reducing product, with $[\alpha]_D^{20}$ +12.5, not fermented by yeast. [d] Analyzed by fermenting with yeast.

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Summary

1. By using the polarimeter and a copper reduction method to follow the partial hydrolysis with invertase of gamma-methylfructoside, the specific rotation of the constituent unstable to the enzyme was found to be $[\alpha]_D^{20}$ −52 ± 2° in water. It was tentatively assumed to be a true methylfructoside of mol. wt. 194.

2. The optical changes suggested that the enzyme hydrolyzed a gamma-fructose derivative possessing the β configuration.

3. Under comparable conditions invertase hydrolyzed sucrose 13.5 times as rapidly as the gamma-fructose derivative and the types of the two hydrolysis–time curves, while not logarithmic, were similar.

4. A fermentation with yeast removed the constituent unstable to invertase from gamma-methylfructoside but left the remainder unchanged in amount and in specific rotation.

5. When the condensation of fructose with acid methyl alcohol was arrested at the point of maximum dextrorotation, approximately 45% of the total non-reducing product was hydrolyzed by invertase and the specific rotation of the remaining 55% was approximately $[\alpha]_D^{20}$ +50° in water, calculated as methylfructoside. These figures, in contrast to the amount of fructose left uncondensed, did not change with wide variation in the conditions of the condensation.

6. When the condensation was prolonged past the point of maximum dextrorotation, only 10–20% of the non-reducing product was hydrolyzed by invertase and the specific rotation of the remainder became strongly levorotatory, due to the production of methylfructosides of the normal less easily hydrolyzable type.

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