neutral to congo paper. The product was recrystallized from alcohol. It is soluble in ethyl and in methyl alcohols, but it is insoluble in water, ether, chloroform, acetone or ethyl acetate; m. p. 221–222° (uncorr.); yield 95%.

Anal. Calcd. for $C_{10}H_{16}O_4N_2$: C, 52.61; H, 7.07; N, 12.29. Found: C, 52.45; H, 7.10; N, 12.25.

Preparation of 5-Isobutyl-5-(β-hydroxypropyl)-barbituric Acid.—Repeated above procedure using 14 g. of 5,5-allylisobutylbarbituric acid, 50 cc. of sulfuric acid and 250 g. of ice. The product is soluble in ethyl and in methyl alcohols, but it is insoluble in water, ether, chloroform, acetone or ethyl acetate; m. p. 216–217°; yield 95%.

Anal. Calcd. for $C_{11}H_{18}O_4N_2$: C, 54.53; H, 7.49; N, 11.58. Found: C, 54.11; H, 7.46; N, 11.62.

Cold concentrated sulfuric acid has no action on 5,5-ethylisopropylbarbituric acid.

Preparation of (II).—Twenty-three grams of (I) in 50 cc. of pyridine and 15 cc. of benzoyl chloride was refluxed for three hours. The product recrystallized from ethyl acetate had m. p. 169–171°. The compound is soluble in ether, alcohol, chloroform or ethyl acetate; yield 70%.

Anal. Calcd. for $C_{17}H_{20}O_5N_2$: N, 8.44. Found: N, 8.43.

Two grams of the product was refluxed with 50 cc. of alcoholic potash (*N*/2) for five hours. The solution was evaporated to about 20 cc. and made acid to congo paper with hydrochloric acid. The crystals which were formed on standing were recrystallized twice from water; m. p. 120–121°; mixed m. p. with benzoic acid 120–121°.

Di-allylation of (II).—11.1 grams of (II) was treated with 8.1 g. of allyl bromide in the presence of finely divided copper and 10% sodium hydroxide according to the method described in British Patent 391,741. The product obtained was a very heavy oil which was purified by vacuum distillation, b. p. (25 mm.) 260°.

Anal. Calcd. for $C_{23}H_{28}O_5N_2$: N, 6.80. Found: N, 6.74. 0.2859 gram absorbed bromine equivalent to 27.66 cc. of 0.1000 *N* bromate solution; required for $C_{23}H_{28}O_5N_2$: 27.58 cc.

Summary

Two 5-alkyl-5-β-hydroxypropylbarbituric acids have been prepared from the corresponding 5-alkyl-5-allylbarbituric acids by addition of sulfuric acid at the double bond of the allyl group and subsequent hydrolysis.

NUTLEY, N. J. RECEIVED JUNE 2, 1934

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

The Analysis of Gamma-Methylfructoside Mixtures by Means of Invertase. III. Behavior of Crystalline Gamma-Methylfructoside in Methyl Alcohol Containing Hydrogen Chloride[1]

BY C. B. PURVES

It has been known for some years that the optical rotation of a methyl alcoholic solution of fructose and hydrogen chloride attained a dextrorotatory maximum when most of the ketose had been changed to non-reducing fructose derivatives of a gamma nature.[2] More recent work[3] indicated that the condensation product included (a) a derivative hydrolyzed by invertase and with a specific rotation, calculated for a methylfructoside, of $[\alpha]_D^{20}$ $-52 \pm 2°$ in water; (b) another non-reducing sirup stable to the enzyme and not more dextrorotatory than $[\alpha]_D^{20}$ $+28°$ and (c) a crystalline gamma-methylfructoside also stable to invertase and with a specific rotation of $[\alpha]_D^{20}$ $+93.0°$ in water and of $[\alpha]_D^{20}$ $+91.6°$ in methyl alcohol. Provided that the reaction was arrested near the point of maximum dextrorotation, no change in the conditions of the condensation seriously affected the relative amounts of the constituents (a), (b) and (c) in the product. In order to discover whether the latter observation indicated the presence in the acid alcohol of an equilibrium among the above three derivatives, a study was made of the behavior of the pure crystalline glycoside (c) when dissolved in the same reagent.

Figure 1, curve A, summarizes the optical data observed, at 20°, when the solvent was 0.0263 normal with respect to hydrogen chloride. The rotation diminished with time in a logarithmic way to a final specific rotation of 22° after which the subsequent decrease was very slow. The unimolecular velocity constant of the primary change, given in minutes and decimal logarithms by $10^4K = 1770$, was no less than 5900 times as large as that determined for the hydrolysis of sucrose with aqueous acid and the same conditions. When the final rotation had been reached (point 1 on the optical curve), the acid methyl alcoholic solution was neutralized and 50% of

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

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

(3) Purves and Hudson. (a) THIS JOURNAL, **56**, 702 (1934); (b) **56**, 708 (1934).

the product was found to consist of the levorotatory glycoside (a) hydrolyzable by invertase (Table I, analysis 1). Although a really satisfactory method of isolating the individual constituents of gamma-methylfructoside mixtures has yet to be discovered, a partial separation is possible by extracting the mixture with ethyl acetate.[3b] In the present case this method was used to recover 36% of the possible yield of the gamma-methylfructoside (c) in a pure, recrystallized condition and with the correct specific rotation of 93°. As the rotation calculated for the entire fraction stable to the enzyme was the same within the experimental error (Table I, analysis 1) and was reproducible on independent occasions, there was little reason to doubt that the entire fraction consisted of the gamma-methylfructoside (c).

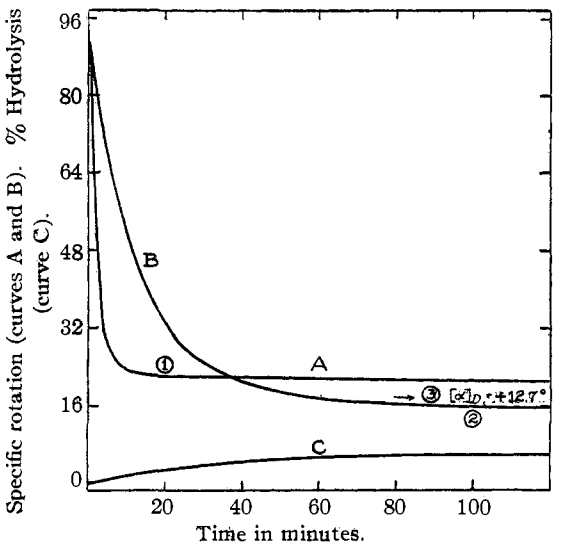

Fig. 1.—Behavior of the crystalline gamma-methylfructoside (c) in anhydrous and in aqueous 0.0263 *N* methy. alcoholic hydrogen chloride at 20°. Curve A, optical rotations in anhydrous alcohol, solution non-reducing throughout; curve B, optical rotations in 1% aqueous alcohol; curve C, % hydrolysis in 1% aqueous alcohol.

TABLE I

ANALYSIS OF MIXTURES PREPARED BY DISSOLVING THE CRYSTALLINE GAMMA-METHYLFRUCTOSIDE IN ACID METHYL ALCOHOL

Anal.	Fructose, %	Glycoside (a), %	Glycosides stable to invertase %	$[\alpha]_D$
1	0.0	50.3	49.7	+94.1
2	5.35	47.6	47.0	+93.0
3	7.5	46.9	45.6	+86.8

In anhydrous methyl alcohol containing hydrogen chloride, therefore, an equilibrium was very rapidly established between nearly equal amounts of the gamma-fructose glycosides (a) and (c) and no evidence was found to indicate the production of any third substance in appreciable quantity.

Experiments were also carried out in which the crystalline gamma-methylfructoside was dissolved in acid methyl alcohol containing 1% of water, the other conditions being the same as before. In this case a partial hydrolysis to fructose was observed (Fig. 1, curve C) while the form of the optical rotation–time curve B was no longer logarithmic and corresponded to a considerably slower reaction. The non-reducing portion of the product had initially the composition already described (analysis 2 and point 2) but underwent subsequent and fairly rapid change (analysis 3 and point 3) when exposed for longer periods to the acid, aqueous methyl alcoholic solvent. As this solvent was similar to that eventually present when fructose was condensed with acid methyl alcohol, an inference can be drawn concerning the composition of the dextrorotatory gamma-methylfructoside mixture so prepared. It can be assumed that as 45% of the non-reducing product consisted of the glycoside (a) unstable to invertase,[3a] another 45% was present as the glycoside (c), although little more than one-fifth of this amount was actually isolated in a crystalline state.[3b] The remainder of the mixture consisted, only approximately, of methylfructoside of the normal ring structure, 2%, and the non-fermentable, uncrystallized derivative (b), 8%, associated with a variable amount of free fructose.

The behavior of the crystalline gamma-methylfructoside in acid methyl alcohol containing water suggested that at least a small proportion of the glycoside (a) might be formed during the hydrolysis of the former with aqueous acid. Invertase, however, caused no change in the copper reduction or optical rotation of a solution containing the partly hydrolyzed glycoside (c) which accordingly had not been appreciably changed through a side reaction to the fructose derivative unstable to the enzyme. The hydrolysis followed a unimolecular course with a velocity coefficient 8.1 times greater than that determined in a parallel experiment with sucrose.

Experimental

Details of the preparative and analytical technique have already been published.[3a,3b]

Action of Anhydrous Methyl Alcoholic Hydrogen Chloride on the Crystalline Gamma-Methylfructoside.—An 0.5672% solution of the pure glycoside in pure, anhydrous methyl alcohol had a dextrorotation of +6.00°V. in a 4-dm. tube. At 20.7° 45 ml. was quickly mixed with 5 ml. of 0.263 *N* methyl alcoholic hydrogen chloride to give an 0.5105% solution of the glycoside 0.0263 *N* with respect to the gas and with a calculated rotation of +5.40° V. After 2, 4, 6, 8, 10, 12, 16, 20, 24 and 29 minutes 2-ml. samples were found to be non-reducing to the Shaffer–Hartmann copper reagent in circumstances where a hydrolysis of 1% of the glycoside present would have been easily detected. The optical observations were made, at 20.7°, in a 4-dm. tube and a final value of $V = +1.30$°V. was assumed in the calculation of the unimolecular velocity constant K which was found to have a value at 20.7° given in minutes and decimal logarithms by $10^4K = 1898 \pm 60$.

TABLE II

OPTICAL CHANGES IN AN ACID METHYL ALCOHOLIC SOLUTION OF THE CRYSTALLINE GAMMA-METHYLFRUCTOSIDE

Min.	V.°	$[\alpha]_D^{21}$	10^4K
0	[5.40]	+91.5	..
1	3.95	66.9	1896
1.5	3.45	58.5	1868
2	3.00	50.8	1912
2.7	2.57	43.5	1909
3.3	2.27	38.3	1878
4	2.05	34.6	1844
5	1.75	29.5	1919
5.75	1.63	27.5	1903
6.8	1.51	25.4	1889
8	1.41	23.6	1964
9	1.38	23.2	1900
10	1.39	23.3	
11	1.35	22.6	
12.25	1.32	22.2	
13	1.33	22.4	$[M]_D^{21}$
16	1.30 (V_∞)	22.0	4270
17	1.30	22.0	4270
20	1.29	21.8	4230
30	1.30	22.0	4270
31	1.29	21.8	4230
135	1.25	20.8	4030
1000	0.97	16.0	3100
1320	0.87	14.4	2800

Another determination, made at 20.0°, gave a value of 1770 ± 60 for 10^4K.

Partial Hydrolysis with Invertase.—The above reaction was stopped by adding $N/100$ caustic soda when the specific rotation had diminished to +22.0°.[4] A solution of the product in water with an acidity of *p*H 4.5 had a dextrorotation of +1.05°V. in a 4-dm. tube. To 20 ml., 10 ml. of the stock invertase solution[3a] was added quickly and the following observations were made on the mixture, kept at 20° in a 4-dm. tube.

Analysis 1.—(See Table I and point 1 on curve A, Fig. 1.) These data when plotted gave a curve with a dextrorotatory maximum (*cf.* enzymotic hydrolysis of the glycoside (a)[3a]). The rotation of the original aqueous solution was changed from +1.05°V. to $(0.125 \times 3/2) + 0.19$°V. by the enzyme, which also caused an increase from 0.004 to 0.208% in the fructose content. These data, together with the value of 0.405% fructose determined after a Herzfeld hydrolysis with aqueous acid, made it possible to calculate[3a] that 50.3% of the product had been hydrolyzed by the enzyme and had a specific rotation in water of −51.5°. The specific rotation computed for the remaining 49.7% stable to invertase was +94.1°.

TABLE III

PARTIAL ENZYMOTIC HYDROLYSIS OF THE EQUILIBRIUM MIXTURE

Min.	V.°	Min.	V.°
0	[+0.70]	9	0.72
1.2	.99	11	.58
1.7	.97	13	.48
2.1	1.00	17	.37
2.7	1.03	20	.28
3.3	1.02	29.5	.17
4	1.00	31.7	.22
4.5	0.98	33	.18
5.3	.96	85	.12
6.3	.92	150	.125 (const.)
7.5	.85		

Recovery of the Crystalline Gamma-Methylfructoside from the Equilibrium Mixture.—The crystalline glycoside, 4.00 g., was allowed to attain the equilibrium specific rotation of +22° in acid methyl alcohol before the solution was neutralized with silver oxide followed by 0.25 ml. of *N* aqueous caustic soda. After reducing the silver-free filtrate to 15 ml., the product was fractionally precipitated by adding 250 ml. of ethyl acetate and evaporating the mixture in a vacuum till the cold supernatant liquid had again become clear[3b] (volume about 60 ml.). Two similar extracts were made before the combined ethyl acetate liquors were concentrated to a sirup, weight 1.95 g.

The glycoside (*c*) crystallized extensively when the sirup was kept free from moisture and the crystals were separated by pressing between filter paper; yield 1.33 g. or 66% of the possible, reduced to 0.72 g. or 36% by recrystallization from 2 ml. of amyl alcohol. The crystals had a specific rotation of +91.7° in water (0.7099 g. in 25 ml. had a dextrorotation of +15.03°V. in a 2-dm. tube), melted sharply at 68° and a mixed melting point with authentic gamma-methylfructoside, m. p. 69°, was not depressed.

Action of Acid Methyl Alcohol Containing 1% of Water on the Crystalline Gamma-Methylfructoside.—The preparation of a methyl alcoholic solution containing 0.5132% of the glycoside, 1% of water and 0.0263 *N* with respect to hydrogen chloride was similar to that adopted with the anhydrous solvent. Optical observations were made, at 20°, in a 4-dm. tube and, concurrently, samples were estimated for fructose by the Shaffer–Hartmann method. The percentage of the ketose, multiplied by 100/0.476, gave the hydrolysis percentage corrected for the change in molecular weight.

Analysis 2.—(See Table I and point 2 on curve B, Fig. 1.) The reaction was stopped in a portion of the above solution after 105 minutes when the specific rotation was

(4) Specific rotations were always determined with sodium light and, unless otherwise stated, at 20°.

TABLE IV

COPPER REDUCTION AND OPTICAL ROTATION OF THE GLYCOSIDE (*c*) DISSOLVED IN ACID METHYL ALCOHOL CONTAINING 1% OF WATER

Min.	V.°	$[\alpha]_D^{20}$	% Fructose
0	[+5.40]	+91.2	<0.002
1	5.12	86.2	
2			< .002
2.7	4.65	78.5	
3.9	4.3	72.2	
5.6	3.91	65.7	
7			.004
7.7	3.47	58.2	
10			.006
10.8	2.95	49.5	
13.1			.007
13.7	2.55	42.7	
17.1	2.19	36.7	
18			.010
19.6	1.98	33.4	
22.3	1.80	30.4	
24.5	1.71	29.0	
25.2			.013
27.8	1.55	26.0	
30.5	1.45	24.5	
33			.016
33.8	1.39	23.0	
37.1	1.30	22.0	
40.3	1.25	21.0	
46.2	1.15	19.3	
53.3	1.10	18.5	
55			.023
60	1.05	17.5	
68.3	1.05	17.5	
79.5	0.98	16.5	
81.7	.99	16.7	
86.7	.95	15.9	
89.8	.95	15.9	
93	.94	15.7	
97			.027
100	.94	15.7	
220	.76	12.7	
224			.037

+15.5° in acid methyl alcohol. The initial rotation of the product dissolved in water, 0.526°V. in a 4-dm. tube, first increased to +1.03°V. in the presence of invertase and then attained the constant value of −0.08°V. An initial reduction of 0.019% fructose increased to 0.176% with invertase and to 0.332% after a Herzfeld hydrolysis. Found: fructose 5.35%; glycoside (*a*) 47.6%, $[\alpha]_D^{20}$ −55°; glycoside (*c*) 47.04%; $[\alpha]_D^{20}$ +92.5° in water.

Analysis 3.—(See Table I and point 3 on curve B, Fig. 1.) Reaction stopped after 224 minutes, $[\alpha]_D^{20}$ +12.7° in acid methyl alcohol. An aqueous solution of the product, with a dextrorotation in a 4-dm. tube of +0.384°V., assumed a value of +0.69°V. in presence of invertase before attaining a constant levorotation of −0.44°V. The initial reduction of 0.038% fructose was increased to 0.259% by invertase and to 0.474% by acid hydrolysis. Found: fructose 7.5%; glycoside (*a*) 46.9%, $[\alpha]_D^{20}$ −55.6°; glycoside (*c*) 45.6%, $[\alpha]_D^{20}$ +86.8° in water.

Hydrolysis of the Crystalline Gamma-Methylfructoside with 0.25 *N* Aqueous Acid.—A 1.7132% aqueous solution of the pure glycoside had a reduction, as fructose, of 0.004% owing to its slight instability toward the alkaline copper reagent. Exactly one-third of the volume of *N* hydrochloric acid was quickly added and the course of the hydrolysis was followed by discharging 2-ml. samples into 10 ml. of 0.055 *N* caustic soda and estimating for fructose by the Shaffer–Hartmann method. The data were corrected for the reduction due to glycoside still unhydrolyzed by subtracting an appropriate fraction of the initial reduction of the diluted solution, 0.003% as fructose.

TABLE V

HYDROLYSIS OF THE CRYSTALLINE GAMMA-METHYLFRUCTOSIDE WITH AQUEOUS ACID

Min.	Fructose, %	Fructose, % (corr.)	10^4K
0	0.003	0.000	..
20	.176	.173	34.1
60	.454	.452	34.5
100	.663	.661	35.1
140	.790	.788	33.6
182	.917	.916	35.0
262	1.05	1.05	35.4
302	1.09	1.09	35.4
Hours			
28	1.19	1.19	..

The theoretical value of 1.192% fructose was taken as *R* in the calculation of the unimolecular velocity constant *K* which was given by $10^4K = 34.7 \pm 1$ (in minutes, decimal logarithms and at 20.2 ± 0.2°). In another experiment a value $10^4K = 32.3 \pm 3$ was obtained by observing the course of the hydrolysis on the saccharimeter.

Examination of the Partly Hydrolyzed Solution.—The hydrolysis of 10 ml. of a 1.420% solution of the glycoside was stopped by adding 5 ml. of *N*/2 caustic soda when the initial rotation of 7.52°V. in a 2-dm. tube had diminished to −1.73°V., corresponding to −1.15°V. in the diluted solution. This value was more levorotatory than the one actually observed after neutralization, −0.91°V., for an undetermined reason which was not connected with a variation due to temperature or solvent in the specific rotation of fructose. After adjusting the reaction of the solution to *p*H 4.5, invertase caused no change in the latter rotation or in the fructose content of 0.543% over a period of nineteen hours although the mixture rapidly hydrolyzed sucrose. No detectable amount of the derivative unstable to the enzyme was therefore present when the hydrolysis of the crystalline glycoside was 61.8% complete. The 0.543% of the fructose in the solution contributed a levorotation, observed in a 2-dm. tube, of −2.845°V.[3a] and the difference of +1.935°V. between this and the observed rotation of −0.91°V. was due to unhydrolyzed glycoside, present in a concentration of 0.361%, corrected for the change in molecular weight. The latter therefore had a specific rotation of +92.7° in water; in excellent agreement with that possessed by the crystalline glycoside (*c*). On another occasion the validity of the calculation was checked by fermenting away the fructose present in a similar partly hydrolyzed solution. The rotation of the

clarified liquid, +3.54°V. in a 2-dm. tube, together with its reduction of 0.608% after a Herzfeld hydrolysis, corresponded to a specific rotation of +93.8° in water.

The author wishes to record his appreciation of the interest which Professor C. S. Hudson has shown in the work and to thank the Chemical Foundation of New York for a Research Associateship.

Summary

1. In the presence of methyl alcoholic hydrogen chloride, the gamma-methylfructoside, $[\alpha]_D^{20}$ +93° in water and stable to invertase, very rapidly gave rise to an equal amount of the gamma-fructose derivative hydrolyzed by the enzyme and with a specific rotation of $[\alpha]_D^{20}$ −52 ± 2°. The reaction was unimolecular and apparently involved no third substance.

2. The above change occurred more slowly when the acid reagent contained 1% of water and in addition a partial hydrolysis to fructose took place.

3. No side reactions were observed during the hydrolysis of the dextrorotatory gamma-methylfructoside by aqueous hydrochloric acid. This unimolecular reaction had a velocity constant 8.1 times as large as that determined for the hydrolysis of sucrose under similar conditions.

WASHINGTON, D. C. RECEIVED JUNE 4, 1934

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

The Analysis of Gamma-Methylfructoside Mixtures by Means of Invertase. IV. Behavior of Sucrose in Methyl Alcohol Containing Hydrogen Chloride[1]

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

The third paper in this series[2] described the equilibrium that was very rapidly established between equal amounts of a dextro-rotatory, crystalline gamma-methylfructoside denoted by (c) and a levorotatory, non-reducing gamma derivative (a) of the ketose when the former was dissolved in dry methyl alcohol containing hydrogen chloride. Although it was desirable to complete the investigation by submitting the latter derivative to the action of the same reagent and so approach the equilibrium from the opposite side, the levorotatory glycoside (a) was not isolated in a condition sufficiently pure to justify its examination. It resembled sucrose, however, in being readily hydrolyzed by invertase and this fact, considered in conjunction with the selective action of the enzyme, made it very probable that the fructose residue in the molecule of both compounds was the same in structure and configuration. Sucrose was therefore regarded in the present instance as the best available substitute for the inaccessible gamma-fructose derivative and the research was continued by examining the reaction occurring between this disaccharide and acid methyl alcohol.

The methyl alcoholic solution, 0.0263 molar with regard to sucrose and 0.0263 normal with respect to hydrogen chloride, corresponded exactly to that previously used in the examination of the crystalline gamma-methylfructoside. When kept at 20° the copper reduction of the sucrose solution rapidly increased from an initial value of zero to a maximum which was due to the liberation in an uncombined state of 94–95% of the theoretical amount of glucose. Thereafter, the reduction slowly diminished (Fig. 1) over a period of days as the aldose recondensed with the acid alcohol to give a non-reducing mixture of gamma-methylglucosides. The correctness of this interpretation of the sucrose reduction curve was confirmed (1) by arresting the reaction near the point of maximum reduction and by isolating from the product 81% of the possible amount of glucose in a pure, crystalline condition, (2) by the fact that the sucrose reduction curve, from the maximum onward for many hours, coincided within the experimental error with the graph depicting the condensation of glucose with acid methyl alcohol in the identical conditions, and (3) by the consideration that any fructose set free would immediately have been recondensed to a non-reducing mixture of gamma-methylfructosides. The latter reaction was 50% complete in six minutes (Fig. 1) while approximately nine minutes were required for a half hydrolysis of the sucrose. These rates of re-

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

(2) Purves, THIS JOURNAL, 56, 1969 (1934).