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The preparation and rate of hydrolysis of glucosides

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THE PREPARATION AND RATE OF HYDROLYSIS OF GLUCOSIDES

A thesis, presented to the Department of Chemistry of Union College, in partial fulfillment of the requirements for the degree of Bachelor of Science in Chemistry, by

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INTRODUCTION

The term glucoside has been applied to those compounds which have the property of supplying, upon hydrolysis, a sugar and one or more other products. Nearly all organic compounds which occur in plants (fruit, bark, roots) are in combination with a sugar. They all resemble the simple methyl glucosides. In many cases, the glucoside form is more soluble than the substance itself.

Naturally occurring glucosides are prepared by extracting from the plant, by means of a solvent such as water or alcohol.

Glucosides are synthesized (generally) from the non-saccharide constituent and acetobromoglucose in the presence of AgO or Ag_2CO_3.

The rates of hydrolysis of glucosides vary widely. They are hydrolyzed in the presence of mineral acids or enzymes, the rate increasing with increasing temperature. The ordinary physicochemical laws governing reversible reactions apply here.

HISTORICAL

Blagoveschnskii (2) found that in the products of synthesis in plants, substances other than proteins play an important role. These are the alkaloids, glucosides, methylated amines, saponins and other substances which form in the secondary conversion of the amino acids which could not be utilized in building up proteins.

Miller (3) proved through the isolation as the tetraacetate, that β-2 trichloroethyl d-glucoside was formed in both roots and leaves of the radish from absorbed chloral hydrate.

Veibel and Frederiksen (4) have reported the rates of hydrolysis, energies of activation (Q), and the constants (B) in the Arrhenius equation $\ln k = (-Q/RT) + B$ for the hydrolysis of several β-glucosides of primary, secondary and tertiary alcohols. A possible mechanism for hydrolysis of glucosides is discussed.

Veibel (5) has shown, in agreement with the results of Heisig (Diss., Breslau, 1937) that a Walden inversion was found for alcoholysis catalyzed by H ion. No Walden inversion occurs with acid hydrolysis in H_2O. According to Lettre

(C.A. 31,7074[2]) for enzymic hydrolysis, an OH group in the enzyme exchanges with an OR group of the glucoside. On becoming free, the enzyme reexchanges OR for OH and completes the hydrolysis. From the ratio

$$\frac{\text{reaction velocity}}{\text{Heat of activation}}$$

it is concluded that the mechanisms of the acid hydrolysis and the enzymatic hydrolysis are the same.

Veibel and Lillelund (6) have shown that comparable velocity constants for 13 glucosides show that steric factors in the aglucone are masked by other factors influencing the rate of hydrolysis or are absent altogether.

EXPERIMENTAL

At the start, it was decided to prepare a simple glucoside, about which adequate literature was available. By preparing this compound it would be possible to familiarize ourselves with the technique required in the preparation, and to check the method of measuring rotation.

It was decided to make α-methyl d-glucoside for this purpose. The compound was prepared in the following manner (7):

Dry HCl (prepared by reacting NaCl and H_2SO_4 and dried by passing thru concentrated H_2SO_4) was passed into 200 g (251 cc; 6.2 moles) of anhydrous methyl alcohol with ice cooling and exclusion of moist air, until an increase of 5 g resulted. This was diluted with 1800 g (2254 cc) of anhyd CH_3OH (this gave a solution of 0.25% HCl). To all this was added 500 g (2.77 mol) of powdered d-glucose. The mixture was refluxed for 72 hours.

This yellow-colored mixture was cooled to 0° and the crystals allowed to form. After allowing the mixture to stand for 2 hours, the crystals were filtered by suction. The product was washed twice with 100 cc portions of absolute methanol. The yield should be 85-100 g (M.P. 165°)

The mother liquor and washings were returned to the flask and refluxed for another 72 hours. This material was then concentrated to 800 cc, chilled to 0° and allowed to stand for 24 hours.

After that period of time, the product was filtered and washed with three 100 cc portions of absolute methanol. The yield should be 110-145 g (M.P. 164-165°)

The mother liquor and washings were concentrated to 300 cc. This concentrate was chilled to 0° and allowed to stand for 24 hours. The product was again filtered and washed.

The product can be recrystallized from absolute methanol. The yield should be from 15-18 g (M.P. 164-165°).

NOTES: Getting a seed crystal was somewhat of a problem. The method which succeeded was to take a few samples from the flask and to dilute one, and concentrate another and leave the third as it was. These were placed in an ice bath until they were cold and then to scratch the side of the test tube with a stirring rod. After some time, the solutions became milky, and then the one of the same concentration as in the flask, showed signs of good crystals. The reaction flask was then

inoculated with these crystals, and after standing 12 hours at 0°, the product had crystalized. All subsequent crystallizations took place readily.

To carry out the problem of checking methods, the rotation of pure dextrose was found, and the calculated specific rotation was checked against a value found in the literature.

Two runs were taken of the actual hydrolysis. The first run was not very successful because of difficulties encountered in the mechanics and technique of thermostating. The second run was successful, and the results were used in calculating the constant (k_r) as given by the formula

$$k_r = \frac{2.3}{t} \log \frac{\alpha_0 - \alpha_\infty}{\alpha_t - \alpha_\infty}$$

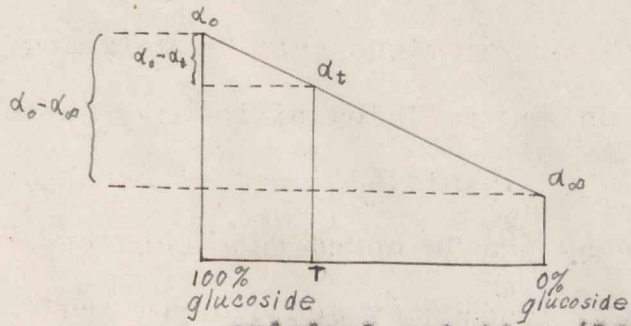
On first looking at the reaction, it appears to be of the second order, but it is actually of the first order. This is true because the concentration of the one constituent remains essentially constant.

It was thought expedient to include a proof justifying the use of the above formula.

In the following proof, it is assumed that the graph drawn is a linear relationship. This was checked experimentally by making up solutions which are the equivalint of those at 0% hydrolysis, 50% hydrolysis and 100% hydrolysis. In doing this, the concentration of acid, amount of glucoside, and amount of dextrose were taken into account. The amount of

alcohol formed in the hydrolysis is negligible.

The results of this work are shown late in the report. We can now continue with the proof.



α_o = original rotation (100% glucoside)

α_∞ = final rotation (0% glucoside)

α_t = rotation after amount (100-T) has hydrolyzed

Slope of graph $k = \frac{\alpha_o - \alpha_\infty}{-100} = \frac{\alpha_o - \alpha_t}{T-100}$

$-100\,k = \alpha_o - \alpha_\infty$ $\qquad$ $k(T-100) = \alpha_o - \alpha_t$

$100 = \frac{\alpha_o - \alpha_\infty}{-k}$ (1) $\qquad$ $(T-100) = \frac{\alpha_o - \alpha_t}{k}$ (2)

Concentration a=100 by definition

$$a = \frac{\alpha_o - \alpha_\infty}{-k}$$

From equation (2) T-100 = amount hydrolysed

a-(T-100) = amount unchanged

(= a-x)

Subtracting (2) from (1),

$$a - x = \frac{\alpha_t - \alpha_\infty}{-k}$$

Substituting for a and a-x in the equation

$$k_r = \frac{2.3}{t} \log \frac{a}{a-x}$$

$$= \frac{2.3}{t} \log \frac{\frac{\alpha_o - \alpha_\infty}{-k}}{\frac{\alpha_t - \alpha_\infty}{-k}}$$

$$k_r = \frac{2.3}{t} \log \frac{\alpha_o - \alpha_\infty}{\alpha_t - \alpha_\infty}$$

After the preliminary work was completed, work was started to determine the effect of different atoms and groups in the (β-ethyl) position of some (β-ethyl) glucosides. The proposed glucosides to be studied were those of

$HO-CH_2-CH_3$

$HO-CH_2-CH_2-OH$

$HO-CH_2-CH_2-Cl$

$HO-CH_2-CH_2-Br$

$HO-CH_2-CH_2-I$

In each case, the tetraacetate has to be prepared first, and then hydrolyzed.

The β-tetraacetylethylglucoside was prepared (8) by mixing 25 grams of glucose pentaacetate with 51 grams of a solution of glacial acetic acid containing 32% of dry HBr (9) by weight. The mixture was allowed to stand at 28° until solution was affected and a deep reddish-brown color appeared (this took about 1 hour). The solution was diluted with 102 cc. of cooled chloroform and poured into 150 cc. of ice water. The layers were shaken together and separated. The water layer was washed three times with 10 cc. portions of chloroform. The total of chloroform washings was washed with sodium bicarbonate until neutral. The bicarbonate was removed by washing with ice water. The chloroform layer was then dried over $CaCl_2$.

The solution was vacuum distilled at 40° to a thick syrup. The syrup was dissolved in 175 cc. of absolute alcohol and shaken in 32 g. of freshly prepared Ag_2CO_3 for a half hour. The silver residue was removed by filtration and washed with ether. The filtrate and washings were evaporated to ½ volume on a steam bath and further by a stream of air. The solution was cooled overnight and filtered. (M. P. 105°) (Absolute alcohol was used for recrystallization.)

The next preparation attempted was that of the glucoside of ethylene chlorohydrin. Since the procedure was not available at the time, an analogy was drawn to the preparation of β-tetraacetylethylglucoside. After two unsuccessful attempts, this work was abandoned, and work on ethylene glycol β-d-monoglucoside tetraacetate was started.

In this preparation, and in all subsequent preparations, acetobromoglucose was used, so it is expedient to include at this point the preparation of that compound (10).

In a 1 L. round-bottomed flask were placed 66 g. of d-glucose and 302 g. (280 cc.) of 95% acetic anhydride. To this mixture were added a few small drops of concentrated sulfuric acid. (Addition of too much acid makes the reaction so vigorous that it is not pos-

sible to control it.) After swirling the mixture for a short time, the reaction started. The swirling was continued throughout the reaction. It was necessary at times to cool the mixture momentarily. The flask was loosely stoppered after the glucose had all dissolved (10-15 min.); the mixture was heated on a steam bath for two hours. About 200 cc. of mixed acetic acid and acetic anhydride were removed by vacuum distillation.

Seventy-five grams (60 cc.) of acetic anhydride was added to the warm, viscous, light yellow syrup. The mixture was warmed slightly, and swirled until the solution appeared homogeneous.

Dry HBr was passed into this solution until a gain in weight of 150 g. was recorded. The flask was sealed with a rubber stopper and allowed to stand over night at 5°.

The hydrogen bromide, acetic acid and acetic anhydride were then removed by vacuum distillation. A water bath (not over 60°) was used to heat the mixture. During the distillation the solution became slightly darker. When no more distillate came over, distillation was stopped, and 250-300 cc. of dry isopropyl ether was added. The mixture was warmed on a steam bath to hasten solution. The hot solution was transferred to a 1 L. Erlenmeyer flask and rapidly cooled to about 45°. The mixture was then allowed to cool slowly to room temperature and then placed in the

refrigerator for two hours (or longer). The aceto-bromoglucose was collected and washed with dry iso-propyl ether and dried in vacuo over $Ca(OH)_2$. (M. P. 87-88°).

Ethylene glycol β-d-monoglucoside tetraacetate was prepared (11) by adding to 40 g. of ethylene glycol 12 g. of acetobromoglucose and 14.4 g. of Ag_2CO_3. The mixture was shaken until no more CO_2 was evolved. Anhydrous benzene (75 g.) was added, and the mixture shaken overnight. The Ag salts were removed by filtration, and the benzene layer was removed from the glycol layer. The glycol layer was further extracted with anhydrous benzene. The benzene layers were cooled and vacuum distilled to a syrup, which crystallized readily. The ethylene glycol β-d-monoglucoside tetra-acetate was recrystallized from water. (M.P. 105-106°; $[\alpha]_D^{23} = -26.3$).

The preparation of tetraacetyl β-d-(β-chloroethyl) glucoside was again tried, this time according to the following procedure (12).

To 25.2 g. (20.8 cc.) of ethylene chlorohydrin were added 6 g. of acetobromoglucose and 7.2 g. of silver carbonate. Carbon dioxide is evolved, and the flask contents were allowed to stand overnight in complete darkness at room temperature. The silver salts were

removed by filtration and washed with hot absolute alcohol. The alcoholic filtrate was then vacuum distilled to remove the excess alcohol and ethylene chlorohydrin. The residue was taken up in hot absolute alcohol, filtered, and on standing, the glucoside started to crystallize out. The crystallization was completed by further cooling the partly crystalline contents. The crystals were filtered and washed well with absolute alcohol. Absolute alcohol was used for recrystallization. (M.P. 114°; $[\alpha]_D^{28} = -21.25$ in acetone).

Tetraacetyl β-d-(β-bromoethyl) glucoside can be prepared in a similar manner, using 39.1 g. of ethylene bromohydrin in place of ethylene chlorohydrin. (M.P. 117.3°; $[\alpha]_D^{27.5} = -20.5$ in acetone) (13).

RESULTS

Proof of Formula

Polarimeter reading (tube containing distilled water) 56.5

Solution--0.5N. with respect to H_2SO_4. Glucoside 10% by weight.

% HYDROLYSIS	OBSERVED ROTATION
100% (after mutarotation)	66.1
50%	77.3
0%	87.8

See graph I

Hydrolysis of α-methyl d-glucoside

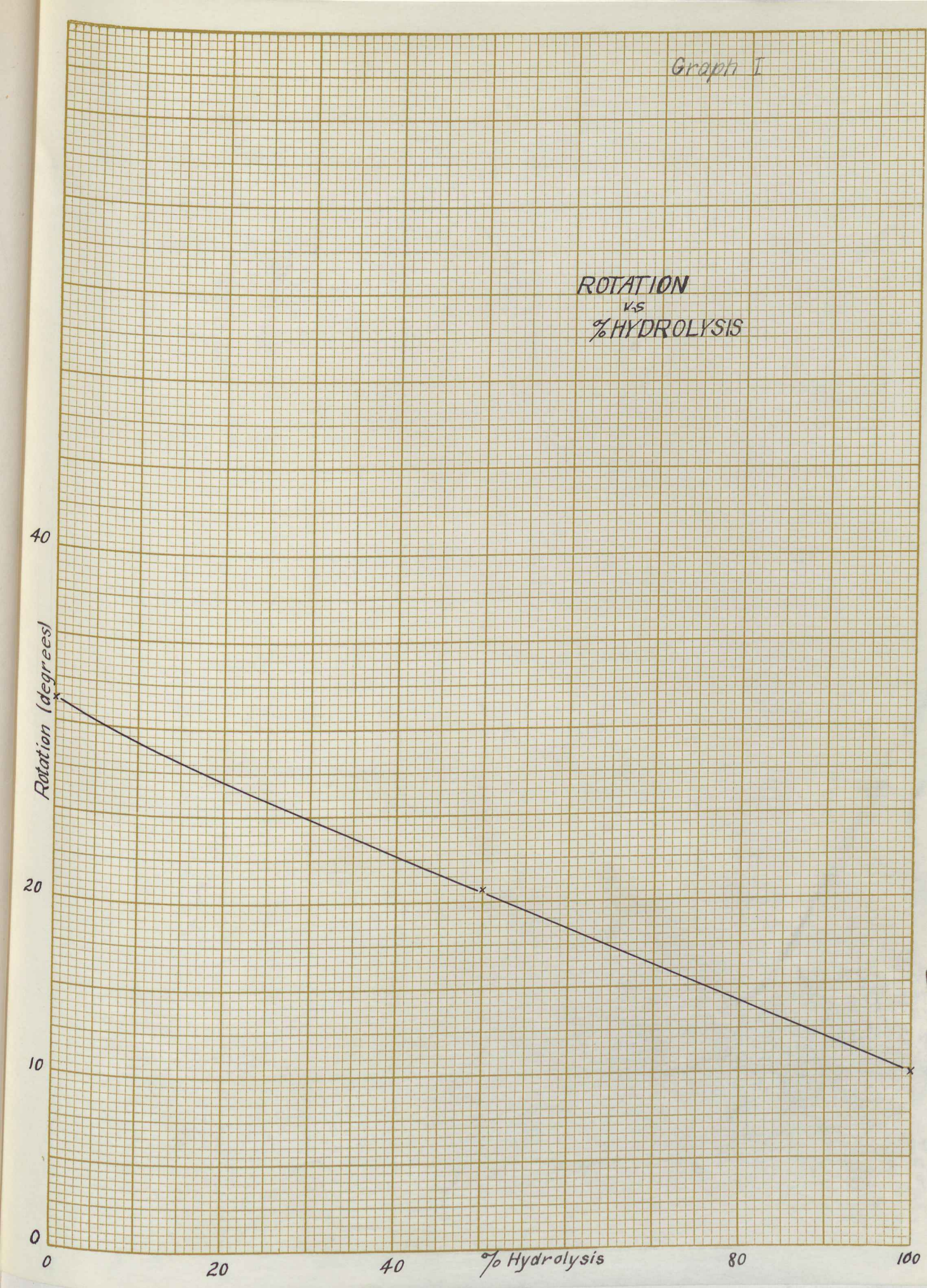
Time (hours)	Rotation
0	31.8
1	31.4
3	30.7
6.5	29.6
10.5*	28.1
15.0	26.8
20.5*	25.1
26.5	23.7
41.5*	20.6
50.5	19.4

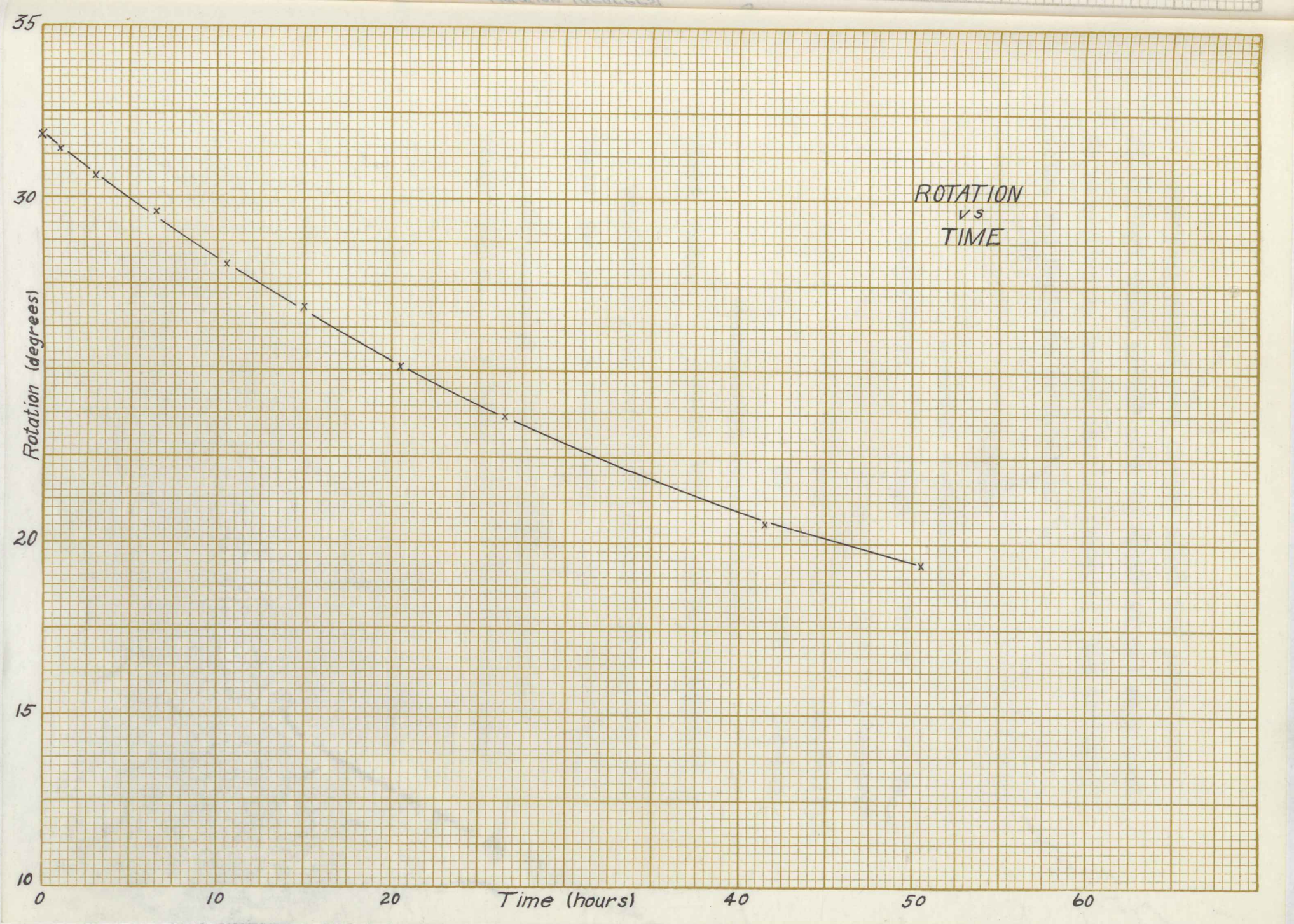
Solution--0.5N. with respect to $H2SO_4$.

Glucoside 10% by weight. Temperature 75°.

* Values used in computing k_r.

Graph I


ROTATION
vs
% HYDROLYSIS
Rotation (degrees)
40
20
10
0
0
20
40
% Hydrolysis
80
100


ROTATION
VS
TIME
Rotation (degrees)
35
30
20
15
10
0
10
20
Time (hours)
40
50
60

Several points were chosen after the graph was drawn, and those marked with an asterisk (*) were used in computing k_r in the derived formula. The average value was found to be 2.973×10^{-4}.

YIELDS

α-methyl d-glucoside

Preparation	Yield	% Yield	M. P.
1.	47 g.	14%	166.5-168°
	22.5		166.5-168.5
2.	36	11%	165-168

β-tetraacetyl ethyl glucoside

Yield 5.3g. % Yield 22% M. P. 104-105.4°

Ethylene glycol β-d-monoglucoside tetraacetate

Yield 4.2 g. % Yield 44% M. P. 103-105°

β-tetraacetyl -d-(β-chloroethyl) glucoside

Yield 2.8 g. % Yield 56% M. P. 112.5-114°

BIBLIOGRAPHY

(1.) E. F. Armstrong--The Carbohydrates and the Glucosides Longmans, Green and Co. 1924

(2.) Blagoveshchenskii--Collection of Papers on Plant Physiol. in memory of K. A. Timiryazer. Acad. Sci. U. S. S. R. Inst. Plant Physiol. 1941, 217-33

(3.) L. P. Miller--Contrib. Boyce Thompson Inst. 12, 359-60 (1942)

(4.) Stig Veibel and Erling Frederiksen--Kgl. Danske Videnskab Selskab, Math.-fys. Medd. 19, No. 1, 38pp (1941) (In French)
C. A. 34, 3774[5] (1940)

(5.) Stig Veibel--Kem. Maanedsblad 20, 253-8 (1939)
Chem. Zentr I, 1503 (1940)

(6.) Stig Veibel and Hanne Lillilund--Z. Physiol. Chem. 253, 55-63 (1938)

(7.) Gilman--Organic Syntheses, Coll. Vol. I John Wiley and Sons, Inc., N. Y. 1932

(8.) J. H. Ferguson--J. Am. Chem. Soc. 54, 4088 (1932)

(9.) C. R. Noller--Organic Synthesis 15, 35 John Wiley and Sons, Inc. 1935

(10.) L. I. Smith--Organic Synthesis 22, 1. John Wiley and Sons, Inc. 1942

(11.) S. Karjala and K. P. Link--J. Am. Chem. Soc. 62 917 (1940)

(12.) H. W. Coles and M. L. Dodds--Pat. 2,252,706
August 19, 1941

(13.) H. W. Coles and M. L. Dodds--Pat. 2,252,706
August 19, 1941