Glycerolysis of Starch and the Molecular Weight and the Viscosity of the Products.

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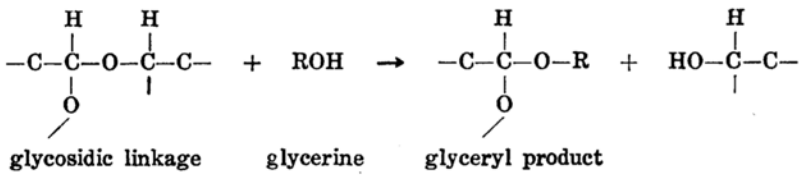
Zulkowski in 1880 obtained degradated starch, soluble in water, by heating starch in glycerine at 190° and by pouring the products into alcohol.[1] Pictet and his collaborators made extensive studies of the glycerolysis of starch and obtained a number of intermediate products (so-called hexosanes).[2] It was, however, shown by Berner and Petersen[3] that these hexosanes are not definite degradation products, containing a certain amount of alcohol and glycerine as impurities, so that the individuality of these products is doubtful. Later Berner and Melhus[4] showed with clearness that glycerine is not only contained as impurity, but also as a constituent of the reaction products, the reaction being represented as follows:



H. Pringsheim gave a detailed description of the products of glycerolysis of starch.[5]

The author has preliminarily examined the procedure of glycerolysis of some varieties of starch changing two conditions, namely time and temperature of the reaction, and has determined the quantity of combined glycerine in the degradation products for the purpose of finding the relation between the glycerine content and the degree of glycerolysis, seeing that Berner and Melhus obtained glyceryl-*d*-glucoside by the total glycerolysis of starch at 180°.

Glycerolysis of Starch. As Zulkowski already noticed, potato starch can be dispersed with ease in hot glycerine, while rice starch does not dissolve so easily. When 8.00 g. dried starch is heated in 60.00 g. distilled glycerine at 180° the appearances of the mixtures were found to change with the progress of time as follows:

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(1) K. Zulkowski, *Ber.*, **13** (1880), 1395; **23** (1890), 3295.

(2) A. Pictet and R. Jahn, *Helv. Chim. Acta*, **5** (1922), 640; P. Castan and A. Pictet, *ibid.*, **8** (1925), 946; A. Pictet, *ibid.*, **9** (1926), 33; A. Pictet and H. Vogel, *ibid.*, **12** (1929), 1220. M. Samec and M. Blinc, *Kolloid-Beihefte*, **48** (1939), 249.

(3) E. Berner and R. Petersen, *Ber.*, **65** (1932), 687.

(4) E. Berner and F. Melhus, *Ber.*, **66** (1933), 1333.

(5) "Die Polysaccharide", Berlin (1931); "The Chemistry of the Monosaccharides and Polysaccarides", New York (1932); Cf. Tollens-Elsner, "Kurzes Handbuch der Kohlenhydrate", 4 Aufl., 570, Leipzig (1935).

Duration of time (hrs.)	Potato starch	Wheat starch	Rice starch
2	Fairly clear	Not transparent	Not soluble
4	Completely clear	,,	,,
8	,,	,,	,,
16	,,	Clear	,,

This shows that at this concentration wheat starch does not easily dissolve in glycerine and that rice starch is more difficultly soluble and the latter was found to get coloured strongly brown. These differences in dispersion of starches according to the nature of plants may probably be due to the difference of the sizes and formes as well as of the inorganic constituent[6] of the grain.

Optical Rotation and Viscosity of the Degradation Products. Optical rotation could not be observed with wheat and rice starches, as clear solution was not obtained from the above products. In the case of potato starch the aqueous solution could be made transparent by mixing the reaction product with potassium hydroxide and its optical rotation was measured. The solution thus prepared contained 0.645% starch and 5.05% glycerine, and the concentration of KOH in the solution was 1/8 normal.

The specific viscosity was measured at 30° with the solution of 5.00 g. reaction product in 45 c.c. H_2O. Both the specific rotation and the specific viscosity diminishes with the duration of glycerolysis, showing that the degradation of the potato starch advances with the time.

Table 1.

Duration of glycerolysis (hrs.)	$[\alpha]_D^{30}$	η_{sp}
2	+165.7°	0.430
4	+162.7°	0.345
8	+148.7°	0.311
16	+145.5°	0.298

In the cases of wheat and rice starches more glycerine is needed to effect dissolution of them. In these cases 5.00 g. dried starch was heated at 180° in 60.00 g. distilled glycerine. The solution for measurement of optical rotation contained 0.422% starch and 6.8% glycerine, and the concentration of KOH in the solution was 1/6 normal. The solution for the measurement of viscosity was made by dissolving 5.00 g. reaction mixture in 40 c.c. H_2O.

The rotation and the viscosity in Table 2 show that the degradation of wheat starch proceeds more slowly than rice starch.

The optical rotation of the acetates of glyceryl potato starch was observed in chloroform at 25°, the results being shown in Table 3.

(6) See M. Samec, M. Minaeff and N. Rouzin, *Kolloid-Beihefte.* **19** (1924), 203; Th. Posternak, *Helv. Chim. Acta*, **18** (1935), 1351.

Table 2.

Duration of glycerolysis (hrs.)	$[\alpha]_D^{25}$		γ_{sp}	
	Wheat starch	Rice starch	Wheat starch	Rice starch
2			1.131	0.819
4	+178.2°	+162.5°	0.911	0.746
8	+176.7°	+148.3°	0.764	0.498
16	+142.0°	+124.6°	0.506	0.498

Table 3.

Glycerolysis		$[\alpha]_D^{25}$	p
Temp.	Time (hrs.)		
170°	6	−163.0°	1.14%
180°	6	+156.8°	1.36 ,,
180°	15	+114.9°	1.14 ,,
180°	29	+103.3°	1.68 ,,

As may be seen from Table 3 the specific rotation of the products falls with higher temperature and with longer duration of glycerolysis.

The Glycerine Content and the Molecular Weight of Glyceryl Starch. The author has prepared various products of glycerolysis of starch and measured their glycerine contents. The preparation of glyceryl starch was carried out as follows: 25 g. wheat starch dried in vacuum at 100° is heated at 180° with mechanical stirring in 100 g. glycerine dried by distilling in vacuum. The reaction product is poured into 80 g. alcohol, the separated glyceryl starch being powdered by rubbing, and filtered through a glass filter by suction. A part of this powder is dissolved in a small amount of water and again precipitated by pouring into alcohol. The precipitate is washed well with alcohol, and dried by heating 10 hours in vacuum at 80°. The rest of glyceryl wheat starch (ca 2.5 g.) is used for acetylation. The glycerolysis product is acetylated at room temperature with 30 c.c. pyridine and 50 c.c. acetic anhydride. After standing over night the reaction product is poured into water, the precipitate dried and purified by precipitating twice from chloroform-alcohol solution. The glycerolysis product obtained by heating for so long as 32 hours is energetically degradated and coloured deeply brown. In this case the glycerine is for the most part distilled off under reduced pressure, the residue is poured into alcohol and then treated as above.

The determination of glycerine was carried out according to the method of Zeisel-Fanto[7]. Instead of concentrated solution of hydrogen iodide of d=1.90, the author used the acid of ordinary concentration (d=1.70). This method really gives values a little lower than the theoretical, but the author has confirmed that it is practically useful. It has been found that glycerolysis products of starch contain a certain amount of combined glycerine even if it is moderately glycerolysed. The glycerine content of the products is shown in Tables 4 and 5.

(7) *Chem. Zeitg.*, **26** (1902), 173; Cf. Schulze, *Chem. Zeitg.*, **29** (1905), 976.

Table 4. Glycerine Content of Glyceryl Wheat Starch.

Product No.	Condition of Glycerolysis		Determination of Glycerine		
	Temp.	Duration (hrs.)	Substance (g.)	AgI (g.)	Glycerine content %
1	180°	4	0.6103	0.0201	1.32
2	180°	8	0 2985	0.0173	2.27
3	180°	16	0.3023	0.0272	3 53
4	180°	32	0.1012	0.1194	7.52

Table 5. Glycerine Content of Acetylated Glyceryl Wheat Starch.

Product No.	Condition of Glycerolysis		Determination of Glycerine		
	Temp.	Duration (hrs.)	Substance (g.)	Agl. (g.)	Glycerine Content %
5	180°	4	0.5988	0.0148	0.97
6	180°	8	0.4014	0.0162	1.58
7	180°	16	0.3024	0.0187	2.43
8	180°	32	0.3024	0.0437	5.67

Potato starch is treated in a similar manner, the results of glycerine determination being shown in Tables 6 and 7.

Table 6. Glycerine Content of Glyceryl Potato Starch.

Product No.	Condition of Glycerolysis		Determination of Glycerine		
	Temp.	Duration (hrs.)	Substance (g.)	AgI (g.)	Glycerine Content %
9	180°	4	0.3974	0.0184	1.82
10	180°	6	0.3063	0.0317	4.06
11	180°	8	0.2208	0.0366	6.72
12	200°	10	0.2390	0.0892	14.6

Table 7. Glycerine Content of Acetylated Glyceryl Potato Starch.

Product No.	Condition of Glycerolysis		Determination of Glycerine		
	Temp.	Duration (hrs.)	Substance (g.)	Agl (g.)	Glycerine Content %
13	170°	6	0.5412	0.0160	1.16
14	180°	6	0.3122	0.0100	1.26
15	180°	8	0.2607	0.0170	2.56
16	180°	15	0.1798	0.343	7.47

As may be seen from Tables 4–7 the glycerine content of the degradation products increases with the rise of temperature as well as with the duration of glycerolysis. This is probably due to the circumstances that by glycerolysis the huge molecule of starch is gradually divided into smaller pieces, a glycerine molecule being attached to the end of a chain molecule. By comparing Tables 4 and 5 with Table 6 and 7 it is seen that the degree of glycerolysis is greater in potato starch than in wheat starch which may be expected from the rotation as well as the viscosity data (Tables 1 and 2).

Now if we assume that the degradation product of starch has a structure, to which end a glyceryl group is attached, we may be able to calculate the chain length of the glyceryl starch by determining the content of glycerine in the degradation product. On the other hand the author has determined the molecular weight of the glyceryl starches by cryoscopy and compared the cryoscopic molecular weight with that determined by the end group method. The results are shown in Tables 8–11, from which it is seen that the molecular sizes determined by the two methods are for the most part in good harmony. For the solvent in cryoscopy water is used with glyceryl starch, and ethylene dibromide C_2H_4Br with its acetylated products. Since water has a small molecular depression and is not a good cryoscopic solvent for starch of large molecular weight, the data in water are in general not very reliable, but ethylene dibromide has proved to be a suitable solvent for cryoscopy of high molecular substances, since it has a very large dissolving power for high molecular substances and is not so sensitive to moisture.[8].

Table 8. Molecular Weight in Glyceryl Wheat Starch.

Product No.	Glycerine Content (%)	M	Cryoscopy			
			Subst. (g.)	Water (g.)	ΔT	M
1	1.32	6990	0.3013	12	0.011°	4250
2	2.27	4050	0.2996	10	0.018°	3100
3	3.53	2610	0.1782	10	0.027°	1230
4	7.52	1224	0.1946	17	0.026°	819

Table 9. Molecular Weight in the Acetates of Glyceryl Wheat Starch.

Product No.	Glycerine Content (%)	M	Cryoscopy			
			Subst. (g.)	C_2H_4Br (g.)	ΔT	M
5	0.97	9500	0.1968	15.26	0.014°	11000
6	1.58	5810	0.1708	16.35	0.027°	6320
7	2.43	3790	0.1500	15.26	0.032°	3690
8	5.67	1620	0.0789	17.05	0.029°	1930

(8) Cf. Y. Tsuzuki, this Bulletin, **3** (1928), 276.

Table 10. Molecular Weight in Glyceryl Potato Starch.

Product No.	Glycerine Content (%)	M	Cryoscopy			
			Subst. (g.)	Water (g.)	ΔT	M
9	1.12	5070	0.3500	16	0.010°	4060
10	4.06	2268	0.5947	10	0.040°	2760
11	6.72	1460	0.1000	11.5	0.013°	1600
12	14.6	629	0.3932	10	0.120°	609

Table 11. Molecular Weight in the Acetates of Glyceryl Potato Starch.

Product No.	Glycerine Content (%)	M	Cryoscopy			
			Subst. (g.)	C_2H_4Br (g.)	ΔT	M
13	1.16	7940	0.7000	26.0	0.045°	7180
14	1.26	7330	0.3029	15.26	0.040°	5960
15	2.56	3590	0.2668	14.75	0.070°	3100
16	7.47	1230	0.2833	16.22	0.158°	1240

The Acetyl Value and the Chain Length. The acetyl value was determined in the following manner:[9] the substance is dissolved in 10 c.c. warm acetone, 15 c.c. N/4–KOH are added, and after standing for 30 minutes, the excess alkali is titrated with N/10–HCl.

The acetyl value thus determined diminishes with the increasing molecular weight of the acetyl starch. This observation is significant with respect to the structure of these products, and indeed the results are to be explained from the idea that a glycerine rest is standing at one end of the chain molecule as follows:

$$\underset{\text{glucose residue}}{C_6H_7O_2(OCOCH_3)_4}-[\underset{\text{glucose anhydride}}{C_6H_7O_2(OCOCH_3)_2}]_n-\underset{\text{glycerine residue}}{C_3H_5O_2(COCH_3)_2}$$

Some typical results will be shown in Table 12, from which it is seen that the observed value of the acetyl content is in good harmony with the value calculated from the above developed formula.

Table 12.

Product No.	Observed			Calculated		
	Mol. wt. by Glycerine Content	Mol. wt. by Cryoscopy	CH_3CO (%)	CH_3CO (%)	n	Mol. wt.
5	9500	11000	45.2	45.10	35	10590
14	7330	5900	45.3	45.26	23	7133
15	3590	3100	45.8	45.73	10	3387
16	1230	1240	47.6	47.64	2	1355

(9) Cf. Y. Tsuzuki, this Bulletin, **3** (1923), 278; **4** (1929), 154.

The Viscosity of the Glyceryl Starch. The author has measured the viscosity of glyceryl starch in water and that of acetylated glyceryl starch in dioxan at 25°, some ones at 20°. The results are shown in Tables 13–16, where p is the concentration, the specific viscosity η_{sp} is equal to the relative viscosity minus one, and C is the concentration in moles of recurring unit per litre. The values of molecular weight determined by the end group method as well as by cryoscopy are quoted in the tables for reference.

Table 13. Viscosity of Glyceryl Wheat Starch.

Product No.	Measurement of viscosity				Molecular weight	
	p (%)	η_{sp}	η_{sp}/p	η_{sp}/C	from Glycerine content	Cryoscopy
1	0.5930	0.0875	0.1476	2.396	6990	4250
2	0.6013	0.0526	0.0875	1.417	4050	3100
3	0.7327	0.0496	0.0677	1.097	2610	1230
4	0.8494	0.0342	0.0403	0.652	1224	819

Table 14. Viscosity of Acetylated Glyceryl Wheat Starch.

Product No.	Measurement of viscosity				Molecular weight	
	p (%)	η_{sp}	η_{sp}/p	η_{sp}/C	from Glycerine content	Cryoscopy
5	0.3844	0.0721	0.1876	5.251	9500	11000
6	0.4054	0.0572	0.1411	3.950	5810	6320
7	0.6206	0.0460	0.0741	2.135	3790	3690
8	0.6368	0.0319	0.0501	1.443	1620	1930

Table 15. Viscosity of Glyceryl Potato Starch.

Product No.	Measurement of viscosity				Molecular weight	
	p (%)	η_{sp}	η_{sp}/p	η_{sp}/C	from Glycerine content	Cryoscopy
9	0.333	0.0786	0.2361	3.735	5070	4060
10	0.489	0.0525	0.1073	1.742	2268	2760
11	0.648	0.0462	0.0712	1.155	1460	1600
12	0.934	0.0360	0.0385	0.626	629	609

Table 16. Viscosity of Acetylated Glyceryl Potato Starch.

Product No.	Measurement of viscosity				Molecular weight	
	p (%)	η_{sp}	η_{sp}/p	η_{sp}/C	from Glycerine content	Cryoscopy
13	0.6277	0.1290	0.2055	5.834	7940	7180
14	0.5593	0.0823	0.1472	4.096	7330	5960
15	0.638	0.0576	0.0903	2.601	3590	3100
16	0.607	0.0223	0.0371	1.069	1230	1240

As may be seen from Tables 13–16, the viscosity of the glyceryl starch η_{sp}/p falls with progressing degradation, i.e. it is in parallel relation with the glycerine content and the cryoscopic molecular weights. In the lower members of these glyceryl starches the molecular weight estimated with the Staudinger's viscosity equation.

$$\eta_{sp}/C = K_m M \qquad (1)$$

coincides with the values of the molecular weight determined by the other two methods, if we assume $K_m = 8.0 \times 10^{-4}$, both in wheat and potato starches. But in the higher members we see that K_m must fall to $3\text{–}5\times10^{-4}$. Several years ago Haworth[10] already observed with dextrins obtained by heating potato starch in glycerine that the molecular weight from viscosity with $K_m=10\times10^{-4}$ is somewhat smaller than that obtained by his end group method. On the contrary he observed in the following cases that the molecular weight from viscosity is several times higher than that determined by his own chemical assay, namely in the cases of waxy maize starch[11] both in acetylated and methylated states and of the potato starch treated with alcoholic hydrochloric acid in the acetylated form, while in the methylated state the two methods gave identical values of molecular weight (26–30 α-glucopyranose units)[12]. Staudinger[13] found such a small value of K_m as 1×10^{-4} by comparing the values of osmotic pressure of disaggregated starch measured by Biltz[14] with his own viscosity measurements. He obtained a still smaller value, $K_m=0.63\times10^{-4}$, in formamide with starch degradated with 2 n–HCl.[15] Staudinger and Husemann[16] obtained $K_m=0.64\times10^{-4}$ in formamide at 20° by studying similarly with wheat starch. In the cases of its triacetyl derivative having the polymerisation degree 600, they found K_m be 1.02×10^{-4} in chloroform. Hirst and Young[17] used $K_m=1.0\times10^{-4}$ in evaluating the molecular weight of acetates of native wheat and rice starches. This value was provisionally obtained from the data of the osmotic-pressure measurements provided by Carter and Record[18] with methylated starches.

Now the author's results appear to be partly in harmony with the results of Haworth and partly with those of Staudinger.

It appears to the author that K_m is not independent on the chain length, as Staudinger has already suggested.[16] It tends to decrease with increasing molecular weight, and indeed in the acetates of glucose and of cellobiose K_m is greater than 10×10^{-4}, but in tetrasaccharide acetate K_m shows a smaller value as 9.0×10^{-4} (in dioxan)[19].

The present investigation concerns with the starch derivatives with

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(10) W. N. Haworth, E. L. Hirst and M. M. T. Plant, *J. Chem. Soc.*, **1935**, 1214.
(11) W. N. Haworth, E. L. Hirst and M. D. Woolgar, *J. Chem. Soc.*, **1935**, 177.
(12) D. K. Baird, W. N. Haworth, E. L. Hirst, *J. Chem. Soc.*, **1935**, 1201.
(13) H. Staudinger and E. Eilers, *Ber.*, **69** (1936), 819.
(14) W. Biltz, *Ber.*, **46** (1913), 1532; *Z. physik. Chem.*, **83** (1913), 683.
(15) H. Staudinger and E. Husemann, *Ann.*, **527** (1937), 195; H. Staudinger, *Naturw.* **25** (1937), 673.
(16) H. Staudinger and E. Husemann, *Ber.*, **71** (1938), 1057.
(17) E. L. Hirst and G. T. Young, *J. Chem. Soc.*, **1939**, 951, 1471.
(18) S. R. Carter and B. R. Record, *J. Chem. Soc.*, **1939**, 664.
(19) H. Staudinger and A. E. Werner, *Ber.*, **71** (1937), 2140.

comparatvely lower molecular weight, so that it is comprehensible that K_m shows a relatively high value. To cover the whole range of widely distributed molecular weight the Staudinger's simple equation does not suffice. The author has, therefore, applied an equation of the modified form:

$$\eta_{sp}/C = K_m M + k \qquad (2)$$

which has already been presented by K. H. Meyer and van der Wyk[20] to express the viscosity of relatively simple high molecular compounds, namely normal paraffines in the range C_{17}–C_{34}. The present author's viscosity data can be expressed fairly well by this equation, the constants K_m and k being shown in Table 17.

Table 17. The Constants K_m and k of Glyceryl Starches.

Glyceryl starch	K_m	k
wheat	4×10^{-4}	0.2
potato	7×10^{-4}	0.2
wheat, acetylated	5×10^{-4}	0.5
potato, acetylated	7×10^{-4}	0.4

It is probable that in the present case a correction term k becomes necessary, since the chain of glyceryl starch is not of uniform constitution, a glyceryl group standing at one end of chain. The higher the molecular weight of the chain the end group becomes less important, so that the correction term k ought to become neglisible with higher polymer-homologues and the simple Staudinger equation holds. It has, however, been remarked that Staudinger's equation requires that the weight average molecular weight be used when it is applied to non-homogeneous polymers,[21] and indeed Flory[22] in recent times derived an equation $[\ln \eta_r/C]_0 = K_w M_w + I$, a modified form of the equation (2), in order to express the relationship between the viscosity-concentration and the weight average molecular weight of nonhomogeneous linear polymers. But the author's glyceryl starch is improved in homogeneity by fractionation and as a matter of fact the ratio M_w/M_n is nearly constant within a given polymer series. Hence the equation (2) is considered to hold in the present case.

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Summary.

(1) Glycerolysis of some varieties of starch has been studied, the progress of which being shown by observing the transparency, the optical rotation and the viscosity of the products. Potato starch is glycerolysed most easily, but wheat starch somewhat difficulty.

(2) With the duration of time the glycerine content of the products of glycerolysis increases, and this content is in parallel relation with the

(20) *Helv. Chim. Acta*, **18** (1935), 1067.
(21) E. O. Kraemer and W. D. Lansing, *J. phys. Chem.*, **39** (1935), 153.
(22) P. J. Flory, *J. Amer. Chem. Soc.*, **62** (1940), 3032.

cryoscopic molecular weight as well as with the acetyl value. Thus it has been shown that a glyceryl group probably stands at one end of the molecular chain of disaggregated starch.

(3) The viscosity measurements of these glyceryl starches indicate that K_m of the Staudinger's equation decreases with increasing molecular weight, and it has been shown that an equation $\eta_{sp}/C=K_mM+k$ expressing the relationship between viscosity-concentration and molecular weight, holds. In this respect wheat starch and potato starch behave a little different from each other.

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