For the preparation of the dimethyl amides, the apparatus was attached to a water pump at the exit side and a current of air mixed with dimethylamine, prepared by dropping an aqueous solution of the amine[11] on solid potassium carbonate, was drawn through the acid during the course of the experiment. The gas was dried by passing it over solid potassium hydroxide. Certain experiments indicated, however, that drying was not essential. The reaction proceeds much faster with dimethylamine than with ammonia.

A study of the physical properties of dimethyl amides, together with methods of preparation and purification, is being continued in this Laboratory by other workers.

Summary

A satisfactory and economical method has been described for the preparation of amides and dimethyl amides from the normal acids of the aliphatic series up to caprylic.

Accurate melting points of the amides from acetic to caprylic have been determined.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

THE POLARIMETRIC REDUCING SUGAR RELATIONSHIPS OF STARCH HYDROLYTIC PRODUCTS RESULTING FROM DIASTATIC ACTION

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The relationship between the specific rotation and copper reducing values of starch hydrolytic products as formed under the influence of diastatic action has been the subject of considerable study. In the early work of Brown and Heron[1] a close relationship was not observed but later work by Brown and associates[2] showed that the hydrolytic products first formed were in a condition of lower optical value and if equilibrium condition of the mutarotating mixture was reached, the rotation assumed the calculated value. Rolfe and Defren[3] claim the same close relationship when the hydrolysis of starch is brought about by acids. Using amylodextrin as the substrate in place of starch, Ford and Guthrie[4] have applied the principle to the estimation of the diastatic power of malt. By determining the change in rotation and multiplying by an appropriate factor

[11] Kindly furnished by the du Pont Co.

[1] Brown and Heron, *J. Chem. Soc.*, **35**, 596 (1879).

[2] Brown, Morris and Millar, *ibid.*, 71, 115 (1897).

[3] Rolfe and Defren, THIS JOURNAL, **18**, 869 (1896).

[4] Ford and Guthrie, *J. Inst. Brewing*, 11, 206 (1905).

they arrive at a Lintner value for the preparation. More recently Gore[5] has employed a very similar procedure with Lintner's soluble starch as the substrate and proposes the method for the quantitative determination of diastatic activity of flour and various other products. In view of the probable complexity and the differences in character of the diastases which are obtained from different sources, as well as the changes in characteristics of the enzyme prepared by different methods of treatment of the same source materials, it is remarkable that the relationship between opticity and reducing power has been as close as it apparently has been. It may be due to the fact that most of the work in this connection has been done with malt diastase. While many investigators have assumed that the enzyme, maltase, is generally absent from malt preparations, the work of Daish[6] has shown that it is of wide occurrence in plant materials. That its presence is very significant has been emphasized by Davis.[7] It is with the consideration of the presence of a glucose-forming enzyme and its effect upon the polarimetric method for the determination of diastase activity that this paper is primarily concerned.

The hydrolysis of starch is usually represented as going through the following steps

$$\text{Starch} \longrightarrow \text{dextrins} \longrightarrow \text{maltose} \longrightarrow \text{glucose}$$

Since the reducing value and optical value of the products between starch and maltose are still matters of much uncertainty, these may be omitted from consideration for the moment and attention directed to the theoretical changes in the polarization and reducing values of the other products. The weight relations are as follows

$$1 \text{ g. of starch} \longrightarrow 1.05 \text{ g. of maltose (or } 1.10 \text{ g. of maltose hydrate)} \longrightarrow 1.1 \text{ g. of glucose}$$

If the specific rotations of the above substances are 199, 138 and 52.5, respectively, then the rotation for 1 g. of soluble starch and its hydrolytic products maltose and glucose if present in 100 cc. and polarized in a 4-dm. tube, Ventzke scale, would be

Starch		Maltose		Glucose
22.9°	⟶	16.8°	⟶	6.66°

and the drop in rotation assuming complete conversion to maltose or glucose would be 6.1 or 16.24° Ventzke, respectively. The reducing changes for 1 g. of starch expressed as apparent maltose hydrate would be

Starch		Maltose hydrate		Glucose
0	⟶	1.10	⟶	$\frac{1.10}{0.61^{8}} = 1.8$

[5] Gore, *J. Assoc. Official Agr. Chem.*, **364** (1923).

[6] Daish, *Biochem. J.*, 10, 49 (1916).

[7] Davis, *ibid.*, 10, 30 (1916).

[8] Average value of the reducing ratio of maltose to glucose from Munson–Walker table.

Hence if 1 g. of starch was completely converted to maltose the Polarization–Reduction ratio (P/R), expressed as Ventzke° 4-dm. tube/Apparent maltose hydrate would be 6.1/1.1 or 5.54 and if converted to glucose would be 16.24/1.8 or 9.04.

A calculation of this ratio from the data of Gore gives the following results

Interval of sample removal	1/2	1	1 1/2	2	2 1/2	3
Ratio for 1/2 hr. interval	5.58	6.00	4.73	6.44	...	4.62
Ratio for total interval	5.58	5.66	5.37	5.71	...	5.65

It is of interest that these values are reasonably close to 5.54, the theoretical value to be obtained assuming complete conversion to maltose.

The object of the experimental work was to determine the polarization–reduction ratio for different amylases and make certain of the general applicability of the method. It was believed that the study might also throw some light on the mechanism of the hydrolysis of starch.

Taka diastase is known to be capable of forming a large proportion of glucose from starch and methods have been devised for the estimation of starch,[9,10,11] assuming formation of maltose and glucose. On account of its glucose forming ability it was chosen as a representative amylase of somewhat different character than that of malt.

Experimental

The method of procedure followed was essentially that outlined by Gore[5] in all instances where the diastase was added in the form of a solution or an extract. In two instances where the diastase yielding material was added in the form of a flour the method of Rumsey[12] was followed. For this method several flasks containing identical quantities of reacting materials were prepared; then after different digestion periods flasks were removed, the solutions clarified and prepared for analysis for apparent maltose. In an article supplementary to one previously cited, Gore[13] recommends buffering the starch substrate so that the P_H is 4.5–5.5. Using as he suggested the equivalent of 10 cc. of both normal acetic acid and sodium acetate per liter of substrate, a P_H value of 4.6 was found. The reaction mixture was buffered in this way in most of the experiments.

Some of the digestion operations were made at room temperature, which was near to 21° at the time. In others the starch solution was brought to a slightly higher temperature, 25 or 39° before the diastase was added. It was then kept at the definite temperature in a thermostat throughout the reaction period. Duplicate determinations of the amount of copper reduced were made by the Munson and Walker method for each interval in all digestion operations and the average values taken. The polarimetric values were the average of six or more readings taken after mutarotation had reached equilibrium.

[9] Davis and Daish, *J. Agr. Sci.*, **5**, 454 (1912–1913).

[10] Horton, *ibid.*, **6**, 152 (1914).

[11] Thomas, THIS JOURNAL, **46**, 1670 (1924).

[12] Rumsey, Morrow's "Biochemical Laboratory Methods," John Wiley and Sons, 1927, p. 287.

[13] Gore, THIS JOURNAL, **47**, 281 (1925).

As substrate material, Lintner's soluble starch supplied by Merck was used in the majority of the experiments. In a few of the later ones a special type of starch prepared as described recently by Gore[14] was employed. However, the concentration was still maintained near 2% instead of 4% as he suggests.

The materials which served as sources of the enzymes were different samples of Taka diastase from Parke, Davis and Company, several commercial malt diastase samples, samples of barley, flour and alfalfa meal.

Partial details of the results of two experiments are given in Table I. These and a number of others are summarized briefly in Table II.

TABLE I

OPTICAL ACTIVITY–REDUCING SUGAR RELATIONSHIPS DURING STARCH HYDROLYSIS UNDER THE INFLUENCE OF ENZYMES

(Experiment 4)

Enzyme, Taka diastase equivalent to 0.05 g. per 100 cc. of substrate; substrate, Lintner's soluble starch, approx. 2%; digestion temperature, 21°

Interval, hrs.	*P* optical value, Ventzke°, 4-dm. tube	*R* Maltose hydrate, g. per 100 cc.	Ratio of changes *P/R* For the interval	Ratio of changes *P/R* From initial observation
0	39.4	0.1389	..	..
0.5	38.2	.4555	3.79	3.79
1	37.4	.7006	3.26	3.56
1.5	36.6	.8225	6.57	4.10
2	35.8	.9677	5.50	4.34
2.5	35.2	1.0602	6.48	4.56
3	34.4	1.1191	13.5	5.09

(Experiment 28)

Enzyme, malt diastase; substrate, "Special" soluble starch, approx. 1.5%; digestion temperature, 25°

Interval, hrs.	*P* optical value, Ventzke°, 4-dm. tube	*R* Maltose hydrate, g. per 100 cc.	Ratio of changes *P/R* For the interval	Ratio of changes *P/R* From initial observation
0	31.42	0.144	..	..
0.5	30.22	.427	4.24	4.24
1	28.42	.657	7.82	5.84
1.5	27.80	.870	2.91	4.98
2	27.16	.989	5.37	5.04
2.5	26.78	1.051	6.12	5.11
3	26.60	1.090	4.61	5.09

Discussion

In the first observations no particular attention was given to maintenance of a constant temperature during digestion, since it was assumed that both polarization and reduction changes would be similarly affected. More careful consideration made it apparent that if the amylase system is composed of several components each with its own particular optimum, the polarization–reduction ratio might vary with the temperature and other factors.

[14] Gore, *Ind. Eng. Chem.*, **20**, 865 (1928).

TABLE II

OPTICAL ACTIVITY–REDUCING SUGAR RELATIONSHIPS DURING STARCH HYDROLYSIS UNDER INFLUENCE OF ENZYMES

Expt.	Diastase	G. per 100 cc.	Digestion temp., °C.	Ratio P/R — Hours 0.5	1	1.5	2	2.5	3	Total change in rotation, 4-dm. tube
2[a]	Taka diastase	0.05	21 ±	2.87	4.17	5.25	6.72	5.72	5.68	6.56
3[a]	Taka diastase	.025	21 ±	2.41	4.03	4.37	4.78	5.13	4.91	5.10
4[a]	Taka diastase	.025	21 ±	3.79	3.56	4.10	4.34	4.56	5.09	5.0
7	Taka diastase	.0062	39	6.87	5.72	6.26	5.84	5.75	..	3.90
8	Taka diastase	.0062	39	3.58	4.69	4.90	4.61	4.12	..	2.74
12	Taka diastase	.0062	39	9.93	7.22	9.87	7.07	5.16	..	3.92
17	Taka diastase	.0062	39	1.65	1.83	2.84	3.11	3.14	..	3.14
21	Taka diastase	.025	25	2.31	2.99	3.18	4.63	5.48	6.02	5.80
22	Taka diastase	.025	25	2.98	3.27	4.67	4.79	4.98	5.24	6.50
23	Taka diastase	.050	25	3.01	3.65	4.25	4.37	5.04	4.31	6.42
24	Taka diastase	.050	25	2.23	2.87	3.07	3.45	3.99	4.07	5.1
26[b]	Taka diastase	.050	25	4.57	4.84	4.75	5.00	5.21	5.16	4.93
27[b]	Taka diastase	.050	25	3.67	4.77	4.70	5.37	5.39	5.62	5.56
5[a]	Malt	.025	21	3.71	5.22	5.67	6.14	7.12	..	4.50
9	Barley flour	.03	39	2.65	3.61	3.61	4.18	4.42	..	2.90
10	Barley flour	.03	39	4.40	8.30	10.50	9.98	7.84	..	2.16
11	Barley flour	.03	39	5.16	4.94	5.44	6.09	6.75	..	4.6
13	Malt	.020	39	6.45	6.00	5.57	5.91	5.44	..	3.95
14	Malt	.020	39	6.87	5.72	6.26	5.84	5.83	..	2.92
18	Malt	.0062	39	4.45	4.26	4.18	3.89	3.46	..	2.07
28[b]	Malt	.050	25	4.24	5.84	4.99	5.04	5.11	5.09	1.82
15	Barley flour	10.0	39	4.67	4.36	4.87	..	..	..	4.99
16	Barley flour	10.0	39	4.79	5.07	5.07	..	..	..	6.3
6	Alfalfa	0.04	39	18.5	13.30	18.4	27.7	21.7	18.2	1.82

[a] No buffer. [b] Special starch.

Considerable difficulty was experienced at times in making the polarimetric observations. The solutions often became quite turbid and the end-point was uncertain. Even the special form of soluble starch prepared according to Gore's directions did not eliminate this trouble. Sometimes the results of duplicate experiments would show almost identical reducing sugar values but markedly different polarimetric changes and *vice versa*. On the whole the results were far from satisfactory and in many respects discouraging. Experiments 15 and 16 using barley flour according to Rumsey's[12] method gave results analogous to the samples of malt diastase. In these experiments the major portion of the change took place during the first half hour interval. If endocellular amylases were present they had very little effect on the P/R ratio.

In conclusion it may be said that although there seem to be differences in the hydrolytic characteristics of malt and Taka diastase materials the effect upon the polarization reduction ratio is such that the variation is probably no greater than other experimental errors in such determinations.

To be of value in throwing light on the mechanism of the hydrolysis of starch the method will have to be improved or give more consistent results for other investigators than it has been possible to attain in this study. It is evident that further attention in this connection must be given to the properties of the hydrolytic products intermediate between starch and maltose.

As can be noted in the data of Gore and those of Table I, the ratio P/R for each successive one-half hour unit interval fluctuates considerably, probably due to error in polarimetric readings, but these differences are smoothed out when the change is calculated from the initial point and some compensation of errors takes place.

In contrast to anticipated results, Taka diastase gave on the whole somewhat lower P/R ratios than malt diastase in the initial stages. This seemed to be more or less independent of the extent of hydrolysis (the latter being represented by the magnitude of the change in rotation given in the last column of Table II). This fact would tend to indicate the formation of reducing substances with relatively little change in optical activity from that of the original starch. It is probable that some glucose is formed from a saccharide unit other than maltose and that it may precede maltose or be formed simultaneously with it, thus giving the lower P/R ratio. Experiments 7 and 12 are out of harmony with others of the Taka diastase group in this respect and had other anomalous features in addition. They are included to indicate experimental variations which sometimes occur without apparent cause. From the results of the experiments completed, temperature, concentration of enzyme and buffering of the solution cannot be said to show any significant effect on the ratio.

Experiment 6 with an extract of alfalfa meal gave a rapid polarimetric change with very little saccharification and hence abnormally high P/R ratios.

Summary

Theoretical consideration of the properties of the final products of hydrolysis of starch resulting from diastatic conversion seemed to indicate that the assumed constant relationship between the polarimetric and reducing sugar values would not hold if glucose as well as maltose resulted from the action of the amylase system. It was further believed that the ratios found might furnish information on the mechanism of the hydrolysis of starch.

Determinations of the ratio have been carried out using Taka diastase as a glucose forming amylase system. The ratios found were different from those anticipated and indicate that the changes in the early stages of hydrolysis are probably unlike those of malt diastase.

While the ratios found were not constant, determinations of diastatic activity based upon the assumption of a constant value will probably not

involve an error more serious than those from other sources in such procedures.

Improvements in the procedure will be necessary to give definite information as to the course of hydrolysis of starch.

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CELLULOSE FUROATE[1,2]

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The increasing industrial importance of cellulose esters presents an attractive field of investigation for new derivatives with desirable properties. One ester which has many desirable properties but which has not been used to any extent because of its excessive cost is cellulose benzoate. The great similarity of furoic acid[3] to benzoic acid made it desirable to prepare cellulose furoate with the hope that it would exhibit the same desirable properties as the benzoate. The recent extensive commercial development of furfural from which the acid can be readily obtained seemed to offer possibilities of obtaining this ester more cheaply than the corresponding benzoate derivative. This ester would be an "all cellulose" ester since furfural can be produced by distilling oxycellulose with dilute hydrochloric acid.

Discussion of Results

The preparation of furoic acid esters of cellulose was accomplished by means of a modified Schotten–Baumann reaction such as had been employed in the preparation of cellulose benzoate.[4] Tetrachloroethane was used as the diluent. Two types of products seemed to be formed as in the case of cellulose acetate, fibrous esters amounting to 65% of the theoretical and analyzing from 2.5 to 3 furoate residues per C_6 unit, and soluble, black, furoylated degradation products analyzing more than three furoate residues. This investigation was restricted mainly to the former product. The fibrous ester was always colored, varying in shade from light yellow to dark brown, and was insoluble in all the usual solvents for cellulose esters.

[1] Abstracted from a thesis by Kenneth A. Kobe presented to the Faculty of the Graduate School of the University of Minnesota in partial fulfilment of the requirements for the degree of Master of Science in Chemical Engineering in June, 1928.

[2] Presented before the Cellulose Division of the American Chemical Society at the 78th meeting, Minneapolis, Minnesota, September 9–13, 1929.

[3] Frankland and Aston, *J. Chem. Soc.*, **79**, 515 (1901); Hill and Palmer, *Proc. Am. Acad. Arts Sci.*, **23**, 188 (1888); Baum, *Ber.*, **37**, 2949 (1904).

[4] Cross and Bevan, *ibid.*, **34**, 1514 (1901); *Chem. News*, **61**, 87 (1890); Cross, Bevan and Beadle, *J. Chem. Soc.*, **63**, 838 (1893); Wohl, *Z. angew. Chem.*, **16**, 285 (1903); Ost and Klein, *ibid.*, **26**, 437 (1913); German Patent 139,669.