but, on standing in the solution, it gradually changed to a more granular condition.

Calculated for $C_{14}H_{14}O_7NAg$: Ag, 25.94. Found: Ag, 26.06.

4-Aminophthalanil, $H_2NC_6H_3\langle{}^{CO}_{CO}\rangle NC_6H_5$.—

Five grams of dimethyl 4-aminophthalate were dissolved in boiling aniline, and the boiling continued for a short time. An excess of acetic acid was added to the cooled solution and then water. By this dilution the anil was thrown out as a yellow precipitate, which was purified by crystallization from alcohol or from glacial acetic acid.

The pure anil crystallizes in long yellowish needles, melting at 205.5° (corr.). It is soluble in alcohol, ethyl acetate, chloroform, or benzene, when hot; slightly soluble in hot water, or cold acetic acid; very difficultly soluble in acetone, ether, carbon disulphide, or naphtha.

Calculated for $C_{14}H_{10}O_2N_2$: N, 11.80. Found: N, 11.93.

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THE OPTICAL ROTATION AND THE DENSITY OF ALCOHOLIC SOLUTIONS OF GLIADIN.

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THE recent application of the polariscope to the estimation of gliadin[1] has rendered desirable some further knowledge regarding the specific rotation of this substance. With many substances optically active in solution, the specific rotatory power changes in a marked manner with changes in temperature, concentration, and nature of the solvent. The experiments given below were carried out by the writer with the view of determining the error that might be introduced into an analytical estimation by such variations.

The gliadin used had been prepared from a hard wheat patent flour by a method used by Osborne and Voorhees.[2] A small sample dried for twenty days in a vacuum over sulphuric acid

[1] H. Snyder: This Journal, 24, 263.

[2] Am. Ch. J. 15, 416, preparation No. 17.

gave 17.65 per cent. and 17.57 per cent. of nitrogen respectively by duplicate determinations made by the Kjeldahl-Dyer method. The standard hydrochloric acid used in these and other determinations had been standardized with silver nitrate in the usual way and checked against pure acetanilide. The proteid was soluble to a perfectly clear solution in dilute alcohol. It is extremely difficult to dry any considerable amount of gliadin within a reasonable length of time, the desiccation being carried out at ordinary temperatures over sulphuric acid *in vacuo*. Most of the material used was therefore merely dried for a few days in the vacuum desiccator and the concentration of the solutions made were found by determining the nitrogen in a given volume. Although the strength of the solution is not fixed as exactly in this way as by weighing out the pure substance, the error introduced is small and possible chemical changes produced in the proteid by removal of the last traces of moisture are avoided.

The polarimetric observations were made with a triple-shadow saccharimeter. In a few cases where the room temperature was considerably above 17.5° C. a small correction was introduced for the increase in the rotatory power of the quartz wedge compensation. Errors due to the difference between the rotation dispersion of quartz and of the solution would probably be much less than the error of experiment and would, of course, affect all observations of a series in a similar way. The temperature of the solution under observation was controlled in the usual way with a jacketed tube. The readings are the means of six observations.

Effect of Variation in Concentration of Gliadin.—The first point considered was the effect on the specific rotation of varying the gliadin concentration. A strong solution in 70 per cent. alcohol was prepared, the polarization determined, and duplicate portions taken for the nitrogen determination. Other amounts were measured off, and by successive dilutions with 70 per cent. alcohol, solutions of different concentrations obtained. The polarizations of these were determined. The following table shows the polariscopic readings (in Ventzke degrees) with solutions of different concentrations. In the third column are given the calculated readings, assuming the gliadin to have a specific rotation $[\alpha]_D^{20°} = -91.3$.

Gram of gliadin in 1 cc.	Observed reading	Calculated reading.
0.05390	—28.38° V.	(—28.38° V.)
0.03601	—18.97° V.	—18.96° V.
0.02406	—12.81° V.	—12.67° V.
0.01607	— 8.54° V.	— 8.46° V.
0.01074	— 5.75° V.	— 5.66° V.
0.007176	— 3.88° V.	— 3.78° V.
0.004795	— 2.60° V.	— 2.53° V.

Similar experiments with solutions in 75 per cent. alcohol gave the following:

Gram of gliadin in 1 cc.	Observed reading.	Calculated reading. $[\alpha]_D^{20^\circ} = -89.2$.
0.01989	—10.12° V.	(—10.12° V.)
0.01329	— 6.70° V.	— 6.76° V.
0.00888	— 4.49° V.	— 4.52° V.

A solution in 80 per cent. alcohol containing 0.01625 gram per cubic centimeter (measured at 45° C.) gave a reading of —7.89° V. at 50° C. Diluted with an equal volume of 80 per cent. alcohol, the reading at 50° C. was —3.92° V.

These results indicate that the specific rotation is little affected by ordinary changes in the gliadin concentration. The apparent slight increase on dilution observed with the solution in 70 per cent. alcohol may have been due to loss of solvent by evaporation when the dilutions were made, although special care was taken to minimize this. As this loss results in an increase in the percentage of water in the remaining solvent, the chance of error is increased, as will be shown later.

Effect of Variation in Temperature.—A gliadin solution in 70 per cent. alcohol, containing 0.0539 gram per cubic centimeter at 20° C. gave the following readings at different temperatures:

Temperature.	Reading.	Specific rotation, $[\alpha]_D$.
20°	—28.38° V.	—91.3
25°	—28.59° V.	—92.4
30°	—28.60° V.	—92.9
35°	—28.53° V.	—93.0
40°	—28.48° V.	—93.1
45°	—28.48° V.	—93.6

In calculating the specific rotations given above the change in length of the glass polariscope tube was so small that it was not taken into account. The expansion of the solution was deter-

mined and found to be approximately regular for this range of temperature, and equal to about 0.1 per cent. per degree. A solution in 75 per cent. alcohol gave the following:

Temperature.	Reading.
20°	—10.12° V.
25°	—10.17° V.
30°	—10.18° V.
35°	—10.10° V.
40°	—10.02° V.
45°	—10.07° V.

A solution in 80 per cent. alcohol of sufficient concentration to give a reading of —7.89° V. at 50° C. was cloudy from separation of gliadin at 45° C. It contained 0.01625 gram of gliadin per cubic centimeter, measured at 45° C. As the solubility of gliadin in strong alcohol increases so rapidly with rising temperature as to indicate a change in phase, solutions in 80 per cent. alcohol, remaining clear when cooled to 25° C., were too dilute to afford measurements of value.

Effect of Varying the Alcohol Concentration of the Solvent.—The preceding data show that with solutions in 70 per cent., 75 per cent. and 80 per cent. alcohol there is a marked increase in specific rotation with increase in percentage of water in the solvent. Below are the values obtained:

Concentration of alcohol in solvent. Per cent.	Specific rotation at 20° C. $[\alpha]_D^{20°}$	Specific rotation at 45° C. $[\alpha]_D^{45°}$	Specific rotation at 50° C. $[\alpha]_D^{50°}$
70	91.3	93.6	....
75	88.2	90.0	....
80	....	....	84.6

As a check a sample of gliadin that had been drying for about ten days was taken and thoroughly mixed. Two portions were then weighed out and dissolved in 70 per cent. and 75 per cent. alcohol, respectively, and made up to known volumes. The apparent specific rotation of the first at 25° C. was —90.3°; that of the second at 40° C., —88.0°. Calculating from the first rotation, taking $[\alpha]_D^{25°}$ —92.4°, the sample contained 2.2 per cent. of moisture. Correcting the second for this we get for the solution in 75 per cent. alcohol $[\alpha]_D^{40°}$ —90.0°. An experiment in which a solution in 70 per cent. alcohol was diluted with sufficient water to bring the alcoholic concentration to about 66 per cent. indicated that a further change in the specific rotation was thus produced, the latter increasing with the increase of water concentration.

As Fleurent[1] has published a method for the approximate determination of gliadin in flours from the density of the alcoholic extract, made under prescribed conditions, it was thought desirable to determine the specific gravity of solutions containing the proteid in different concentrations. The following table shows the density of solutions of different concentrations in 70 per cent. alcohol. They were taken with a pycnometer.

Gram of gliadin per cc.	Specific gravity 20°/4°.
0.05390	0.8865
0.03601	0.8815
0.02406	0.8777
0.01607	0.8745
0.01074	0.8729
0.007176	0.8717
0.004795	0.8702
0.000000	0.8686

It would appear from this that even an approximate determination of gliadin in alcoholic solutions by means of the density would be quite difficult, as the change in specific gravity for a given change in the gliadin concentration is not especially large, and the disturbing effects of evaporation which would decrease the alcohol concentration of the solvent, absorption of moisture from the flour and variation in temperature could easily vitiate the results.

SUMMARY.

The specific rotation of gliadin in 70 per cent. to 75 per cent. alcohol is practically independent of the gliadin concentration.

With 70 per cent. to 80 per cent. alcohol it decreases with increase in the alcohol concentration.

Increase in temperature between the limits 20–45° C. produces a slight increase in the specific rotation.

The change in density in gliadin solutions for such differences as would be met with in flour analysis would allow rather a narrow margin for experimental error.

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[1] Abs. in J. Soc. Chem. Ind. 20, 941, from Compt. rend. 132, 1421.