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Henry Semple Blair[a]; Peter James Watt[a]; Ronald Cromie[a]
[a] Department of Industrial Chemistry, The Queen's University of Belfast, Belfast, Northern Ireland

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A Spectroscopic Investigation of Phenylhydrazine Derivatives of Amylose

HENRY SEMPLE BLAIR, PETER JAMES WATT, and RONALD CROMIE

Department of Industrial Chemistry
The Queen's University of Belfast
Belfast, Northern Ireland

ABSTRACT

The paper describes an investigation of the preparation and properties of amylose phenylosazone and phenylhydrazone. Under the conditions used, amylose phenylosazone was not formed quantitatively and considerable degradation of the amylose occurred. The phenylhydrazone, however, can readily be prepared in quantitative yield and the polymer remains undegraded. Solutions of amylose phenylhydrazone in dimethylsulfoxide may be used to determine the number-average molecular weight of amylose samples.

INTRODUCTION

The usual method for the determination of the number-average molecular weight of amylose is by osmotic pressure measurements on derivatives of the amylose, commonly the fully acetylated amylose in chloroform solution [1]. However, owing to the possibility of gel

formation with the higher molecular weight fractions [2], this method may present some difficulty. Other methods involve the use of amylose methyl ether or amylose nitrate, but degradation is possible during the preparation of these derivatives [3]. A number of methods involving end group determination have been described [4-6]. The various reducing value methods, e.g., alkali number, ferricyanide number, and 3,5-dinitrosalicylic acid method, are unsatisfactory as none of these gives an absolute evaluation of terminal aldehyde groups in amylose [7]. This is due to degradation of the reducing end group of the polysaccharide chain in the alkaline medium used, giving rise to the production of other reducing substances which interfere with the determination. Other end group methods described include periodate oxidation [8], enzymatic hydrolysis [9], and polarography [10].

Recently a spectroscopic method for the determination of $\overline{M}_n$ of cellulose and hydrocellulose has been described [11]. This is based on the formation of a phenylosazone derivative involving the reducing end group of the cellulose. Owing to the similarity of the chemical structures of cellulose and amylose, it appears that this method might be applicable to the determination of $\overline{M}_n$ of amylose.

EXPERIMENTAL

Materials

Unless otherwise stated, the reagents used were "Laboratory Standard" grade and were used as received from the manufacturers. In preliminary experiments amylose, B.D.H. "Biochemical Reagent" was used as obtained. Where indicated, the amylose was purified by two fractionations with n-butanol [12] to remove traces of amylopectin.

Ultraviolet and Visible Spectra

Ultraviolet and visible spectra were recorded with Unicam SP500 and SP800 spectrophotometers using matched 10 mm stoppered silica cells. "Spectroscopic" grade dimethylsulfoxide was used as a solvent.

Determination of the Molecular Weight of the Amylose Samples

The molecular weight of the amylose samples was determined viscometrically in 1 M potassium hydroxide [13]. The potassium

hydroxide was purged with nitrogen for 15 min before the addition of the amylose sample, and the solution kept under nitrogen during the determination.

The flow times of the amylose solutions were determined using a suspended level dilution viscometer fitted with a sintered glass filter disk of porosity 2. The viscometer was supplied by Polymer Consultants Ltd., who stated that the error arising from the neglect of kinetic energy corrections is small for water, becoming vanishingly small for more viscous solvents. As a result, no kinetic energy corrections were made.

The limiting viscosity numbers $[\eta]$ were calculated using the method of least squares and the limiting viscosity number-molecular weight relationship used was that proposed by Cowie and Greenwood [13]:

$$DP_n = 7.4[\eta]$$

where $[\eta]$ is measured in g^{-1} cm^3.

Volatile Content

The volatiles contained in the amylose samples were determined by heating samples to constant weight in a vacuum oven at 100°C.

D-Arabino-Hexose Phenylosazone (D-Glucose Phenylosazone)

This was prepared by the method of Richtmeyer [14] mp 207-209°C (lit. 208-209°C).

Amylose Phenylosazone

Method 1

Amylose (1 g) was pasted with phenylhydrazine (15 cc) and 10% acetic acid (100 cc) added, and the solution heated on a steam bath. The product was recovered quantitatively by adding the reaction solution to 200 cc of ethanol and centrifuging. The precipitate was purified by extracting for 12 hr with ethanol in a Soxhlet extractor and dried under vacuum at room temperature.

Method 2

The water in Method 1 was replaced by a 1:1 water:ethanol mixture.

Method 3

The water used in Method 1 was replaced by 2-methoxyethanol and the reaction carried out under nitrogen.

Method 4

This method was similar to Method 3 except that the reaction was carried out in an atmosphere of air.

D-Mannose Phenylhydrazone

D-Mannose (5 g) was dissolved in water (25 cc) and phenylhydrazine (10 g) added and the mixture shaken. The solution was left to stand overnight and the D-mannose phenylhydrazone separated by filtration and purified by extraction with methanol and water, mp 198-200°C (lit. 199-200°C) [15].

Amylose Phenylhydrazone

Phenylhydrazine hydrochloride (10 g) and sodium acetate trihydrate (10 g) were boiled in water (60 cc) and the solution filtered to remove any undissolved tars. The solution was allowed to cool to room temperature and amylose (2 g) pasted in a little methanol added. The product was recovered quantitatively by adding an excess of ethanol (120 cc), centrifuging, and extracting the solid for 12 hr with ethanol in a Soxhlet extractor. The product was dried under vacuum at room temperature.

RESULTS AND DISCUSSION

Phenylosazones

Amylose phenylosazones were prepared by Method 1 which is similar to that of Blair and Cromie [11] for cellulose and M_n for each sample determined viscometrically. It was found that as the time of reaction was increased, the absorbance at 390 nm increased to a constant value after 30 hr (Table 1). It is apparent from viscosity measurements that degradation is occurring during phenylosazone formation ($[\eta]$ for a typical amylose sample changed from 207 to 58 over 11 hr). This degradation may be due to both oxidation and hydrolysis [16]. Comparison of ϵ values of amylose phenylosazones (for a sample after an 11-hr reaction, ϵ = 7660) with ϵ for D-glucose phenylosazone show

TABLE 1. The Absorbances of 1% Solutions of Amylose Phenylosazones in Dimethylsulfoxide at 390 nm (ϵD-Glucose Phenylosazone = 20,400)

Time of reaction (hr)	$E^{1\%}_{1\ cm}$
0	0.00
2	0.66
4	0.78
6.5	0.90
8.5	0.94
15.5	1.08
20.5	1.82
23	1.61
28	2.10
35	2.11
42	2.10

that phenylosazone formation is not quantitative. In an attempt to eliminate the oxidative degradation, various modifications were made to the reaction conditions. Aqueous ethanol was used to prevent the amylose from dissolving in the reaction mixture; however, under these conditions the sample was still degraded. The use of 2-methoxyethanol as a solvent in the reaction for the formation of phenylosazones has been described [14] but when used in this reaction, either under nitrogen or in air, mixtures of phenylosazone and phenylhydrazone were obtained. As the formation of a phenylosazone requires oxidative conditions [17], it would appear that in order to obtain a phenylosazone some oxidative degradation of the amylose is inevitable.

Phenylhydrazones

The phenylhydrazones of amylose were prepared as described above. It should be noted that the reaction is carried out at room temperature and at a pH of about 6.2, and also that the amylose and its phenylhydrazone are largely insoluble in the medium used. These

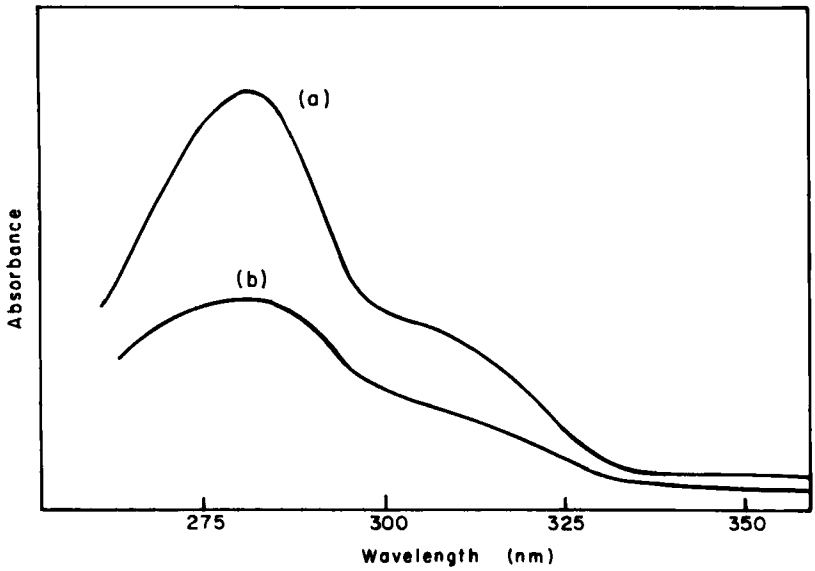

FIG. 1. Absorption spectra of (a) D-mannose phenylhydrazone and (b) amylose phenylhydrazone in DMSO.

factors reduce the possibility of both oxidative and hydrolytic degradation. With increasing time of reaction the absorbance at 280 nm (due to the phenylhydrazone group) increases, reaching a maximum after about 100 hr (the absence of an absorption band at 390 nm indicates that the phenylosazone is not being formed). Even after this period there was no appreciable reduction is viscosity (Table 2). ϵ for the phenylhydrazone of a twice-fractionated amylose was found to be 21,050 at 280 nm, and this compares well with 19,950 for D-mannose phenylhydrazone. (This was taken as a model because of its stability and ease of isolation in a pure form. O'Donnell and Percival [18] have shown that ϵ for D-glucose phenylhydrazone is the same as that for D-mannose phenylhydrazone, Fig. 1.)

CONCLUSION

From the work reported it would appear that the phenylhydrazone of amylose is a suitable derivative to use for the determination of $\overline{M}_n$ by a spectroscopic method.

TABLE 2. The Absorbances of 1% Solutions of Unfractionated Amylose Phenylhydrazones in Dimethylsulfoxide at 280 nm

Time of reaction (hr)	$E^{1\%}_{1\ cm}$	$[\eta]$ ($g^{-1}cm^3$)
0	0.00	196
24	0.73	-
48	0.84	-
72	1.00	-
96	1.11	192
168	0.90	-

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