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Spectroscopic Investigation of Amylose 1-Phenylflavazole Derivatives

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

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Spectroscopic Investigation of Amylose 1-Phenylflavazole Derivatives

HENRY SEMPLE BLAIR and PETER JAMES WATT

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

ABSTRACT

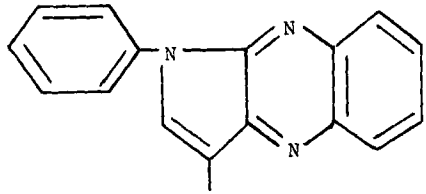
Amylose 1-phenylflavazole has been prepared and its ultraviolet absorption spectrum determined in DMSO solution. Five samples of amylose 1-phenylflavazole were prepared in which different times of reaction were used. Although the polymer was hydrolyzed during the reaction, the molar extinction coefficient of each sample was equal to that of maltose 1-phenylflavazole, and so the number-average degree of polymerization of the initial sample of amylose can be determined by extrapolation. This value was in close agreement with that obtained by viscometry.

INTRODUCTION

Kjolberg [1] and Banks [2] have shown the presence of long chain branches terminated by a nonreducing end group within the amylose molecule. Methods of determination of the number-average molecular weight of amylose by assay of the nonreducing end groups will therefore lead to an average branch length within the sample. We

have recently described a method for the accurate determination of the number-average molecular weight of amylose [3] (by condensation of phenylhydrazine with the reducing end group of the molecule and then determining the phenylhydrazone by ultraviolet spectroscopy) which overcomes the above difficulty.

In an effort to extend the method we have examined the reaction of the reducing end group of amylose with o-phenylenediamine and phenylhydrazine to form the 1-phenylflavazole (I).



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EXPERIMENTAL

Materials

Unless otherwise stated, the reagents used were laboratory standard grade and were used as received from the manufacturers. Amylose, B. D. H., biochemical reagent grade, was purified by two fractionations with n-butanol [4] to remove traces of amylopectin.

Ultraviolet and Visible Spectra

Spectra were determined as previously described [3].

Molecular Weight of Amylose

The molecular weight of amylose was determined as previously described [3].

Maltose 1-phenylflavazole

This was prepared by the method of Neumüller [5]. The product was dissolved in hot 1-propanol, treated with a little decolorizing charcoal, filtered hot, and allowed to cool slowly to room temperature. After isolation of the solid it was recrystallized from glacial acetic acid; mp 254-256°C (lit. [5] mp 262-264°C).

Amylose -1phenylflavazole

Amylose (1 g) suspended in a little methanol was dispersed into boiling oxygen-free water; o-phenylenediamine (1.08 g), phenylhydrazine hydrochloride (7.2 g), and glacial acetic acid (2.4 g) were added to the mixture heated under nitrogen on a steam bath. After heating for the required period the product was recovered by adding the solution to ethanol (200 ml) and centrifuging. The product was dissolved in dimethyl sulfoxide (DMSO), filtered through a sintered glass crucible, reprecipitated with ethanol, recovered, and further purified by treatment with ethanol in a Soxhlet extractor for 24 hr.

RESULTS AND DISCUSSION

The ultraviolet and visible absorption spectrum of maltose 1-phenylflavazole in DMSO was determined and is shown in Fig. 1.

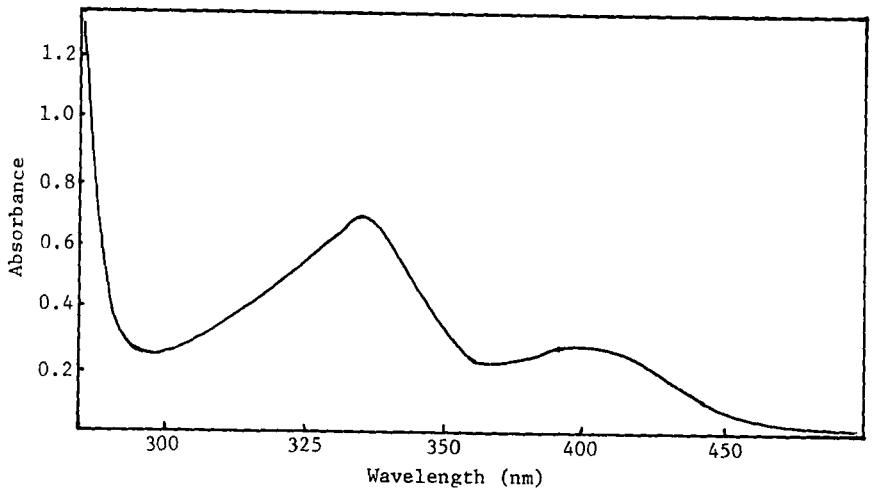

FIG. 1. Absorption spectrum of maltose 1-phenylflavazole.

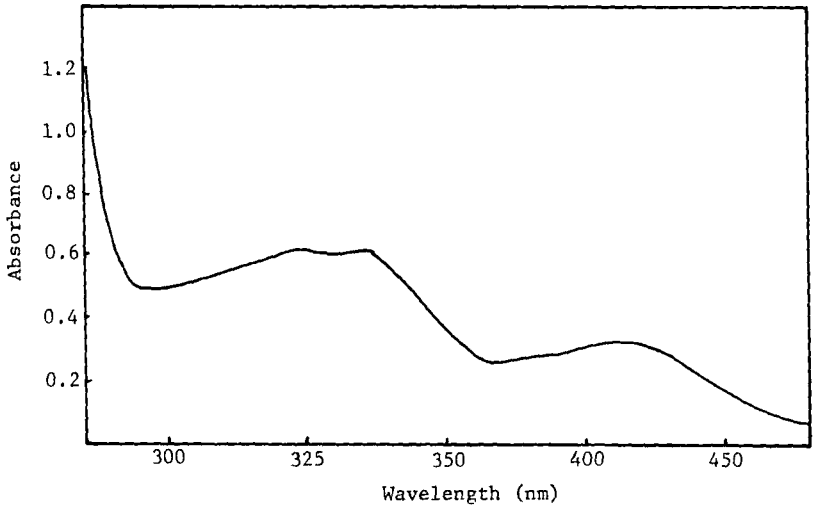

FIG. 2. Absorption spectrum of amylose 1-phenylflavazole.

The spectrum is characterized by maxima at 336 and 407 nm. (A third absorption band close to the cut-off point of the solvent was not recorded).

Amylose was treated with phenylhydrazine hydrochloride and o-phenylenediamine, while nitrogen was bubbled through the solution, under the conditions of Neumüller [5]. The absorption spectrum of the resulting product is shown in Fig. 2. In addition to the maxima at 336 and 407 nm, a further peak appeared at 323 nm which is not present in the spectrum of the analogous maltose compound. After a further purification by precipitation from DMSO and extraction with ethanol, the ratio of the optical densities of the amylose 1-phenylflavazole peaks at 323 and 336 nm remained unaltered, showing that the offending absorbing species was part of the amylose 1-phenylflavazole, not merely a contaminant which had evaded removal during the purification process. Evidence that the interfering species was not involved in the reaction with the reducing end group is that molar extinction coefficient for the amylose 1-phenylflavazole in DMSO was 3.96×10^3 at 407 nm which was equal to that of maltose 1-phenylflavazole ($\epsilon = 3.92 \times 10^3$) within the limits of experimental error. The contaminant, therefore had no effect on the determination of the reducing end groups of the amylose molecule and could be disregarded.

TABLE 1. Effect of Time of Preparation of Amylose 1-Phenylflavazole on Extinction Coefficient of its Solution in DMSO

Time (hr)	$E^{1\%}_{1\ cm}$ at 407 nm
1.0	0.146
2.5	0.462
5.0	0.847
6.5	1.078
9.0	1.629
13.0	1.889

To determine the time necessary for complete reaction of the reducing end groups of amylose with o-phenylenediamine and phenylhydrazine hydrochloride, samples were treated for periods of up to 13 hr, and the extinction coefficients for 1% solutions ($E^{1\%}_{1cm}$) in DMSO determined. The results are shown in Table 1 and Fig. 3.

Over the range of reaction periods there was no evidence of the

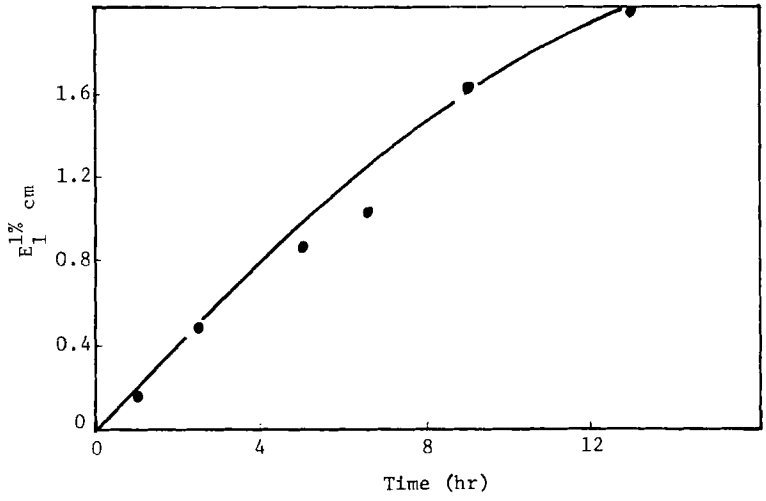

FIG. 3. Plot of $E^{1\%}_{1\ cm}$ of DMSO solution of amylose 1-phenylflavazole against time of formation.

TABLE 2. Effect of Time of Preparation on the Molar Extinction Coefficients and Degree of Polymerization of Amylose 1-Phenylflavazole in DMSO Solution[a]

Time (hr)	$DP_n \times 10^{-2}$	$\epsilon \times 10^{-3}$ at 407 nm
4	5.12	3.95
5	3.48	3.84
6	3.71	3.87
7	3.31	3.96
8	2.53	3.88

[a] ϵ for maltose 1-phenylflavazole is 3.92×10^3.

value $E^{1\%}_{1\ cm}$ reaching a constant value, indicative of the reaction reaching completion. In addition, the number-average molecular weight of the sample treated for 6.5 hr had dropped from an initial value of 2.51×10^5 to 4.00×10^4. The pH of the reaction medium was found to be 3.2, indicating that degradation of the polymer was due to acidic hydrolysis. (A sample of amylose 1-phenylflavazole was prepared by using a reaction medium buffered to pH 5.8, but the absorption spectrum of the product was complex showing that in addition to 1-phenylflavazole, phenylosazone and phenylhydrazone were also being formed in significant quantities).

Several amylose 1-phenylflavazoles were prepared at pH 3.2, and their molar extinction coefficients in DMSO at 407 nm were determined (Table 2). This shows that, although the polymer was being hydrolyzed, new undamaged end groups were forming and reacting completely to form a 1-phenylflavazole which could be determined and used to calculate the number-average degree of polymerization of the hydrolyzed amyloses.

It is generally agreed that the homogeneous acid hydrolysis is first order in respect of the number of unhydrolyzed linkages. A number of workers [6-8] have treated the acid hydrolysis of amylose and cellulose statistically; however Greenwood and co-workers [9, 10] and Whelan [11] have shown in practice that the number-average degree of polymerization of acid-hydrolyzed amylose is inversely proportional to the time of hydrolysis. The results of such a plot for the amylose 1-phenylflavazoles of Table 2 are shown in Fig. 4 and Table 3.

An extrapolation of the plot to zero time by use of the method of

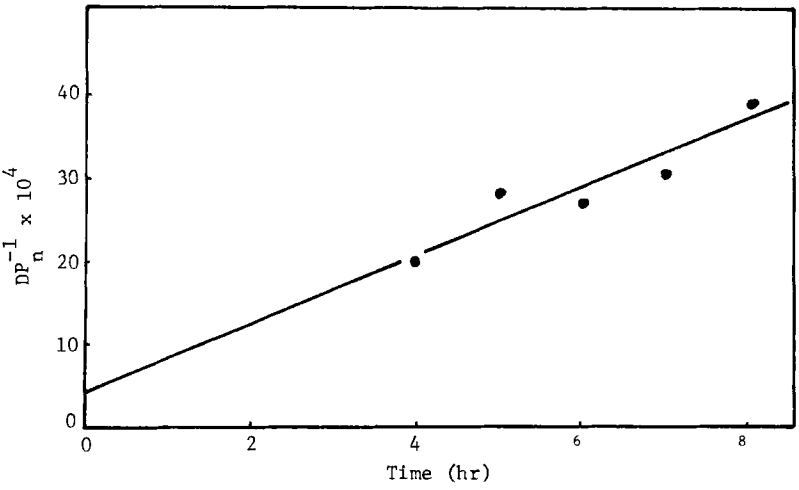

FIG. 4. Plot of reciprocal of $\overline{DP}_n$ against time of formation of 1-phenylflavazole.

TABLE 3. Effect of Time of Preparation of Amylose 1-Phenylflavazole on $\overline{DP}_n$ of the Sample

Time (hr)	$\overline{DP}_n$	$DP_n^{-1} \times 10^4$
4	512	19.53
5	348	28.74
6	371	26.95
7	331	30.21
8	253	39.53

least squares resulted in a value of 2.34×10^5 for the number-average molecular weight of the amylose sample, which is in good agreement with the value obtained by viscometry, ($\overline{M}_n = 2.51 \times 10^5$).

CONCLUSION

The molar extinction coefficients of a series of amylose 1-phenylflavazoles at 407 nm in DMSO were equal to those of maltose 1-phenylflavazoles. The number-average degree of polymerization of the amylose 1-phenylflavazoles could therefore be calculated, and hence by extrapolation a value for the initial degree of polymerization could be determined. This value was in close agreement with that obtained by viscometry.

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