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Spectroscopic Investigation of Reducing Endgroup Derivatives of Hydrocellulose

H. S. BLAIR and R. CROMIE,* *Department of Industrial Chemistry, Queen's University, Belfast, Ireland*

Synopsis

A series of reducing endgroup derivatives of hydrocellulose have been prepared and their suitability for spectroscopic endgroup analysis examined. Of the compounds examined, the semicarbazide, thiosemicarbazide, hydroxylamine, benzimidazole, ethylacetoacetate, nitrophenylhydrazine, and phenylosotriazole derivatives were for one reason or another unsuitable. The phenylhydrazine-*p*-sulfonic acid derivative has properties which would make it suitable for endgroup analysis, but unfortunately neither the glucose nor the cellobiose derivative could be prepared. The 1-phenylflavazole derivative, however, appears to be very suitable for endgroup analysis.

INTRODUCTION

Recently, spectroscopic methods have been described for the determination of molecular weights of hydrocellulose[1] and amylose,[2] based on the formation of phenylhydrazine derivatives of the reducing endgroups of the polysaccharide molecules. The present work reviews the suitability of a range of other reducing endgroup derivatives for spectroscopic endgroup analysis of hydrocellulose.

EXPERIMENTAL

Materials

The reagents used were of laboratory standard grade and were used as received from the manufacturers.

Ultraviolet and Visible Spectra

Ultraviolet and visible spectra were recorded with the Unicam SP 800 and SP 500 spectrophotometers, using matched stoppered silica cells throughout.

Preparation of Cellulose Solutions in Cadoxen

The cadoxen solvent, hydrocellulose samples, and cellulose solutions in cadoxen were prepared as previously described.[1]

* Present address: School of Physical Science, Ulster College, The Northern Ireland Polytechnic, Jordanstown, Newtownabbey, Co. Antrim, Ireland.

Determination of Molecular Weight of Hydrocellulose Samples

The molecular weights of the hydrocellulose samples were determined viscometrically using the limiting viscosity number–molecular weight relationships proposed by Marx-Figini and Schulz,[3] as previously described.

Hydrocellulose *p*-Nitrophenylhydrazine Derivative

Method 1. A suspension of hydrocellulose (1 g) was refluxed with *p*-nitrophenylhydrazine (15 g) suspended in 10% acetic acid (100 cm^3) for 16 hr. The product was collected on a sintered glass filter, and washed with 10% acetic acid, water, and finally ethanol. The product was then extracted for 24 hr with ethanol in a Soxhlet extractor and air dried.

Method 2. A suspension of hydrocellulose (1 g) in water (10 cm^3) was mixed with a solution of *p*-nitrophenylhydrazine (15 g) in ethanol (60 cm^3) and glacial acetic acid (15 cm^3), and the mixture was refluxed for 16 hr. The product was isolated and purified as in method 1.

Method 3. Hydrocellulose (1 g) was refluxed for 16 hr with *p*-nitrophenylhydrazine (1 g) in a reaction medium consisting of water (70 cm^3) and 2*M* acetic acid (10 cm^3), and sodium acetate was added to buffer the reaction medium to pH 6.0. The reaction product was isolated and purified as in method 1.

Method 4. Similar to method 3, except that ethanol was used instead of water.

Additional Methods. The reaction conditions used in methods 3 and 4 were used with variations in the buffer concentration to give a pH range from 1.5 to 7.0. In no case was a pure derivative obtained.

Hydrocellulose Phenylhydrazine-*p*-sulfonic Acid Derivative

Hydrocellulose (1 g) was suspended in a solution of phenylhydrazine-*p*-sulfonic acid (1 g) in water (100 cm^3). Sodium acetate was added to buffer the solution at pH 6.0. The reaction mixture was refluxed for 18 hr, and the product was collected on a sintered glass filter, and washed with water and then ethanol. The product was then extracted for 10 hr with ethanol and air dried.

Hydrocellulose Phenylosotriazole

A portion of hydrocellulose phenylosazone (1 g) was refluxed in 250 cm^3 copper(II) sulfate solution for 20 hr. The product was collected on a sintered glass filter, washed with distilled water, and then extracted for 6 hr in a Soxhlet extractor with ethanol and air dried.

Hydrocellulose *p*-Bromophenylosotriazole

A portion of hydrocellulose (1 g) was suspended in water (15 cm^3). Bromine (1 cm^3) was added drop by drop, with shaking. The flask was stoppered and left standing at room temperature for 72 hr. The product was col-

lected on a sintered glass filter, washed with distilled water and acetone, and then air dried.

Hydrocellulose 1-Phenylflavazole

Hydrocellulose (1 g) was suspended in water (45 cm^3), and *o*-phenylenediamine (1.08 g), phenylhydrazine (5 cm^3), 2*M* hydrochloric acid (25 cm^3), and glacial acetic acid (2.5 cm^3) were added. The reaction mixture was adjusted to pH 5.8 by addition of sodium acetate. The reaction mixture was outgassed with nitrogen for 20 min and then heated under reflux for 22 hr. The product was collected on a sintered glass filter and washed with ethanol and then water. It was then extracted with ethanol for 24 hr and air dried.

Hydrocellulose *o*-Phenylenediamine Derivative

This was based on the preparation of D-glucose benzimidazole.[4] Hydrocellulose (1 g) was treated with a mixture consisting of copper(II) acetate (5.5 g) and *o*-phenylenediamine (3.42 g) dissolved in 100 cm^3 of 4% acetic acid. The mixture was heated at 53°C for 24 hr and the product isolated on a sintered glass filter.

The product was washed with successive portions of distilled water for 24 hr, then extracted with ethanol for 24 hr, and finally air dried.

Hydrocellulose Semicarbazide Derivative

This was based on the preparation of D-arabinohexosulose bis(semicarbazone).[5]

Hydrocellulose (1 g) was suspended in a solution of aniline (3 g), semicarbazide hydrochloride (6.15 g), and sodium acetate (7.5 g) in 2*M* acetic acid (5 cm^3), water (8 cm^3), and ethanol (50 cm^3), and the mixture was refluxed for 20 hr. The product was filtered off, washed with water and ethanol, extracted with ethanol for 6 hr, and finally air dried.

Hydrocellulose Ethylacetoacetate Derivative

This was based on the preparation of ethyl 2-(D-arabinotetrahydroxybutyl)-5-methyl-4-furoate.[6]

Hydrocellulose (2 g) was treated with anhydrous zinc chloride (5 g), ethylacetoacetate (5 g), and 96% ethanol (20 cm^3) under reflux for 20 hr. The hydrocellulose derivative was isolated by filtration, washed with water and ethanol, extracted for 10 hr with ethanol, and finally air dried.

D-Glucose phenylosotriazole[7]; D-glucose *p*-bromophenylosotriazole[8]; D-glucose *p*-nitrophenylhydrazone[9]; D-glucose *p*-nitrophenylosazone[10]; D-glucose 1-phenylflavazole[11]; D-glucose benzimidazole[4]; D-arabinohexosulose bis(semicarbazone)[5]; and ethyl-2-(D-arabinotetrahydroxybutyl)5-methyl-4-furoate[6] were prepared by reported methods. The melting points agreed with those in the literature.

RESULTS AND DISCUSSION

In an earlier paper,[1] we reported the use of hydrocellulose phenylosazone in 50% cadoxen solution for the determination of the molecular weight of hydrocellulose by UV spectroscopy. The present work describes investigation of the suitability of a range of other reagents for reducing endgroup determination. The molecular weight may be calculated using the expression

$$\epsilon = \frac{A\bar{M}_n}{cl}$$

where ϵ is the molar extinction coefficient, $\bar{M}_n$ is the average molecular weight, c is the concentration of the solution (g/l.), and l is the path length of the cell (cm).

To calculate ϵ, a derivative of known molecular weight must be available. For convenience, this ideally should be a model compound, e.g., for hydrocellulose it should be D-glucose or D-cellobiose. It can, however, be a sample of the hydrocellulose whose $\bar{M}_n$ has been determined by some other means. In the light of these requirements, a series of derivatives of hydrocellulose was examined to assess their suitability or otherwise for determination of the reducing endgroups.

Semicarbazide, Thiosemicarbazide, Hydroxylamine, and Ethylacetoacetate Derivatives

These were prepared as described. Their absorption spectra in 50% cadoxen solution did not show any characteristic absorption bands above 250 nm. All attempts to prepare the bis(semicarbazone) derivative were unsuccessful.

Benzimidazole Derivative

D-Glucose benzimidazole is readily formed, and in 50% cadoxen solution its absorption spectrum shows maxima at 280 nm and 245 nm. When hydrocellulose is treated under the same reaction conditions, the product has no characteristic absorption above 250 nm, and it was concluded that hydrocellulose does not form a benzimidazole derivative.

p-Nitrophenylhydrazine Derivative

In all cases, mixtures were obtained, and the reagent proved to be unsuitable for endgroup analysis.

Phenylosotriazole and *p*-Bromophenylosotriazole Derivatives

These were prepared as described, and their Cadoxen solutions showed single absorption bands at maxima of 270 nm and 276 nm, respectively. However, in neither case did complete conversion of the osazone to the osotriazole occur.

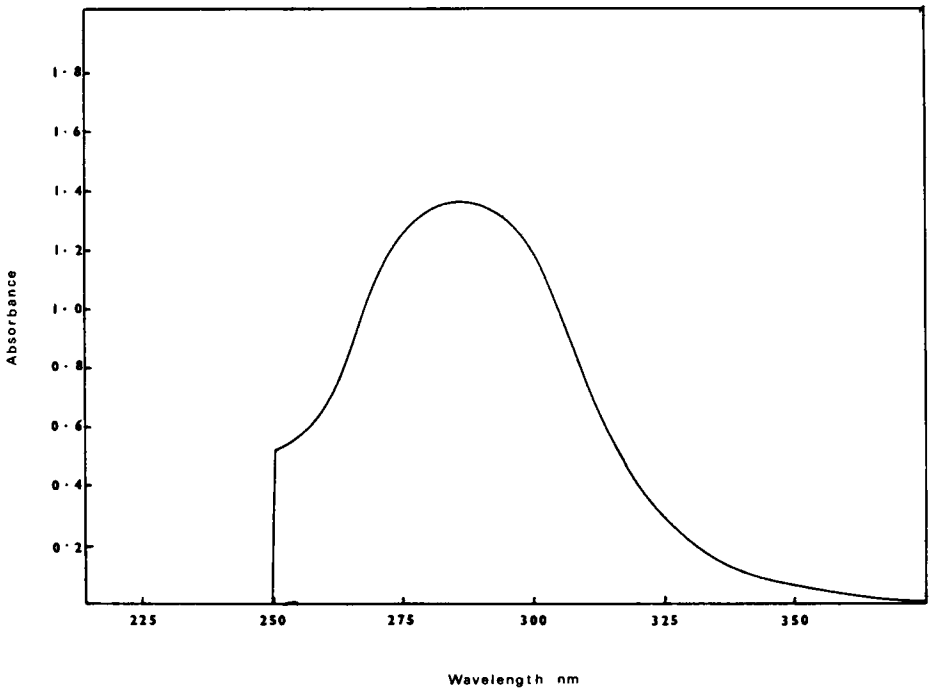

Fig. 1. Absorption spectrum of hydrocellulose phenylhydrazine-*p*-sulfonic acid derivative in cadoxen solution.

TABLE I
Reaction Times and Extinction Coefficients for Hydrocellulose Phenylhydrazine-*p*-sulfonic Acid Derivative

Reaction time, hr	Extinction coefficient k, (328 nm)
2	1.36
5	2.69
8	3.42
12	3.93
18	4.25
22	4.24

TABLE II
Molecular Weights and Molar Extinction Coefficients for Hydrocellulose Phenylhydrazine-*p*-sulfonic Acid Derivatives

Sample	Molecular weight × 10^{-4}	$\epsilon_{282\ nm}$
1	55.60	16,374
2	16.78	16,431
3	14.50	16,168
4	11.60	16,219
5	10.13	16,454
6	7.24	16,103
7	4.18	16,229
8	3.91	16,262

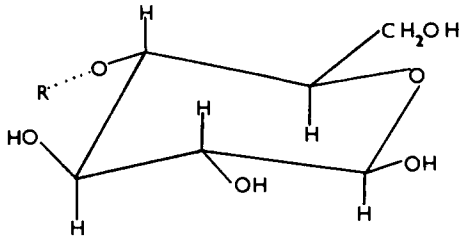

Fig. 2. Steric hindrance of chain substituent R— on C_3 hydroxyl group of terminal D-glucose unit.

Phenylhydrazine-*p*-sulfonic Acid Derivative

This derivative of hydrocellulose is readily prepared and has a well-defined absorption band (λ_{max} 282 nm) in 50% cadoxen solution.

Seven samples of a hydrocellulose were refluxed with the phenylhydrazine-*p*-sulfonic acid mixture for varying times up to 30 hr and their ultraviolet absorption spectra determined in 50% cadoxen solution. Figure 1 shows the absorption spectrum of a freshly prepared solution of this derivative after 16 hr of refluxing, and Table I shows the increase of absorbance of the derivative with time of refluxing. It is apparent that the reaction is substantially complete after 14 hr; but to ensure complete reaction, the samples were refluxed for 18 hr.

As with the absorption spectrum of hydrocellulose phenylosazone[1] in 50% cadoxen, that of the phenylhydrazine-*p*-sulfonic acid derivative changes

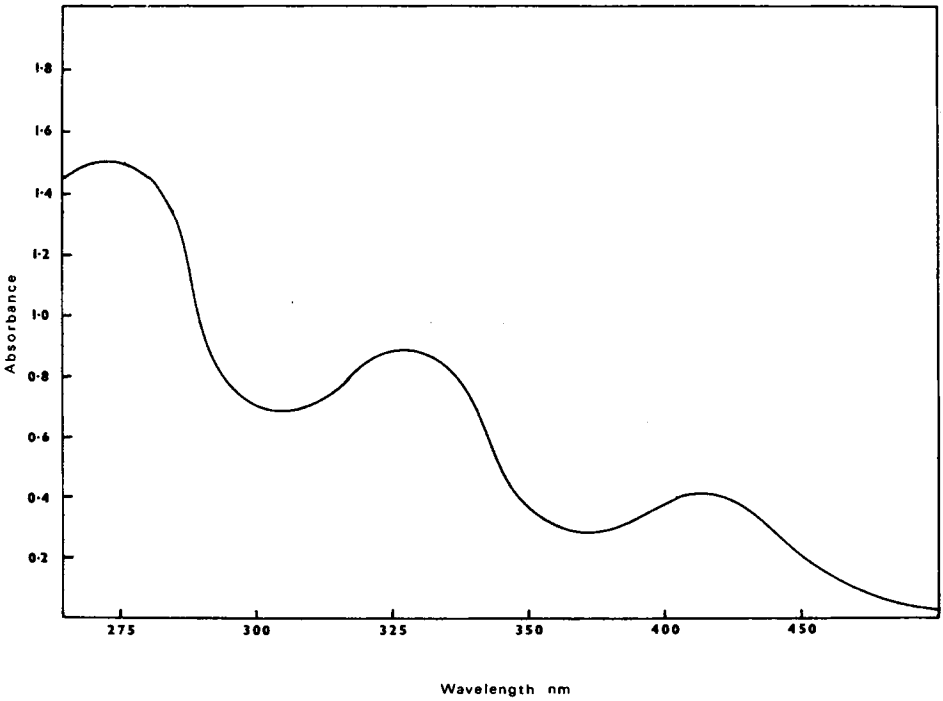

Fig. 3. Absorption spectrum of hydrocellulose 1-phenylflavazole derivative in cadoxen solution.

slowly with time. However, with the rigorous exclusion of oxygen, the solution was found to be stable, and no change in absorbance at 282 nm was observed.

Table II shows the molar extinction coefficients of eight derivatives of hydrocellulose of varying molecular weights: these are well within the limits of experimental error.

In order to obtain the molar extinction coefficient of a sample of hydrocellulose phenylhydrazine-*p*-sulfonic acid, it is necessary to know the absorbance of a derivative of known molecular weight. Unfortunately, all attempts to prepare the phenylhydrazine-*p*-sulfonic acid derivative of D-glucose or D-cellobiose were unsuccessful, nor were records of their preparation found in the literature. This makes it necessary to determine the molar extinction coefficient of the derivative of a sample of hydrocellulose of known molecular weight. This condition limits the use of phenylhydrazine-*p*-sulfonic acid as a reagent for endgroup analysis of hydrocellulose.

1-Phenylflavazole Derivative

The 1-phenylflavazole derivative of D-glucose is characterized by absorption maxima at 267 nm, 335 nm, and 410 nm. The molar extinction coefficient in 70% propanol/water solution is unaffected by substituents at C_4 and C_6, and the solutions were found to obey the Beer-Lambert law.[14]

It was expected that the terminal glucose residue at the reducing end of the hydrocellulose chain would form a 1-phenylflavazole derivative as it has C_2 and C_3 hydroxyl groups free. However, there could be some steric hindrance to the reaction due to the presence of the bulky chain substituent R— on C_4 as this reduces the reactivity of the C_3 hydroxyl group (Fig. 2).

Nevertheless,the derivative does form, and its spectrum in 50% cadoxen solution is similar to that of D-glucose 1-phenylflavazole in cadoxen, and is characterized by maxima at 276 nm, 328 nm, and 414 nm (Fig. 3): the spectrum does not change with time. Complete reaction occurred after about 18 hr of treatment with phenylhydrazine and *o*-phenylenediamine at pH 5.8 at 80°C. A further 4 hr did not increase the extent of the reaction. Table III shows the molar extinction coefficients of the 1-phenylflavazole derivative of eight samples of hydrocellulose, measured at 328 nm and 414 nm in 50% cadoxen solutions. The average value of ϵ at 414 nm is 5,635, and this compares

TABLE III
Molecular Weights and Molar Extinction Coefficients for Hydrocellulose 1-Phenylflavazole Derivatives

Sample	Molecular weight × 10^{-4}	$\epsilon_{414\ nm}$	$\epsilon_{328\ nm}$
1	55.60	5,645	12,464
2	16.78	5,673	12,621
3	14.50	5,598	12,479
4	11.60	5,610	12,498
5	10.13	5,703	12,523
6	7.24	5,638	12,484
7	4.18	5,629	12,686
8	3.91	5,581	12,536

favorably with that of D-glucose 1-phenylflavazole (5,684). However, at 328 nm, the average value is 12,536, and this is considerably higher than that of the D-glucose derivative (ϵ = 12,040).

This may be due to a small amount of an impurity not completely removed by ethanol extractions, or to scatter. If the method of Morton and Stubbs[15] is used to eliminate such unwanted absorption, the recalculated value (ϵ = 12,141) for the samples differs only slightly from that of the D-glucose derivative, and the derivative thus appears to be suitable for spectroscopic endgroup analysis.

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References

1. H. S. Blair and R. Cromie, *J. Appl. Polym. Sci.*, **16**, 3063 (1972).
2. P. Watt, Ph.D. Thesis, Queen's University, Belfast, Ireland.
3. M. Marx-Figini and G. V. Schulz, *Makromol. Chem.*, **54**, 102 (1962).
4. C. Edward, C. Heath, and S. Roseman, *Methods in Carbohydrate Chemistry*, Vol. II, R. L. Whistler and M. L. Wolfrom, Eds., Academic Press, New York, 1963, p. 138.
5. H. El-Khadem, G. H. Labit, and M. A. Nashed, *Carbohydrate Res.*, **3**, 509 (1967).
6. E. S. West, *J. Biol. Chem.*, **74**, 561 (1927).
7. R. M. Hann and C. S. Hudson, *J. Amer. Chem. Soc.*, **67**, 735 (1944).
8. H. El-Khadem and Z. M. El-Shafei, *J. Chem. Soc.*, 3117 (1958).
9. A. Gereces, L. Somogyi, and A. Foti, *Acta Chim. Hung.*, **34**, 113 (1962).
10. A. Van Ekenstein, J. J. Blanksma, *Seits. Ver. Deutsch. Zuckerind.*, 190 (1904).
11. H. Ohle and M. Hielscher, *Ber.*, **74**, 13 (1941).
12. P. Nordin and M. Doty, *Science*, **134**, 112 (1961).
13. R. A. Morton and A. L. Stubbs, *Analyst*, **71**, 348 (1946).

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