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Colorimetric determination of the molar substitution of 2-hydroxybutyl and related starch ethers*

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The molar substitution (MS) of 2-hydroxyalkyl starch ethers, e.g. 2-hydroxybutyl starch, can be determined spectrophotometrically. The principle involves a dehydrative–hydrolytic generation of *n*-butyraldehyde from the 2-hydroxybutyl starch ether substituent in concentrated sulfuric acid. The resultant hydrolyzate containing the aldehyde and allylic species of the substituent is complexed with 2,2-dihydroxyindan-1,3-dione (ninhydrin) in bisulfite giving a colored complex the absorbance intensity of which can be determined by a spectrophotometer at 470 nm. From the maximum intensity observed the concentration of butyraldehyde in the sample is derived by interpolation on a standard curve. The percentage of 2-hydroxybutane in the sample is calculated by comparison of the concentration of substituent to that of the hydrolyzed starting material. The MS is then calculated from the per cent substituent.

INTRODUCTION

Corn starch is one of the most abundant raw materials in the USA. It has found many uses in food and non-food products alike. A particularly exciting development for this surplus material is the potential of increasing new industrial uses as a component in the formulation of composites in plastic blends. Such plastics would not only provide a better economic position for corn growers but also an environmentally sound approach to alleviating part of the present crisis posed by non-degrading synthetic plastics. To make the highly hydrophilic starch polymer compatible with the highly hydrophobic synthetic polymer to produce blends of useful properties, some of the possible approaches would be: (1) use of plasticizers such as ethylene-co-acrylic acid copolymer (EAA) (Otey *et al.*, 1980), or other complexing agents; (2) grafting the starch to the synthetic polymer chain; or (3) modifying the starch by derivatization so that it would readily become compatible with the synthetic polymer in the blend.

In the last case it would be helpful to know the properties of the derivatized starch so as to tailor the resulting blend to specific needs. The first step in such a process is a knowledge of the degree of substitution (DS) of the starch derivative. The DS of starches derivatized with monofunctional substituents has usually been estimated by hydrolytic procedures on known amounts of derivative using appropriate reagents. Upon extraction and weighing of the recovered substituent, the percentage of substituent and the degree of substitution on the anhydroglucose unit can be calculated.

For substituents derived from oxirane addition, complexities arise. While the above hydrolytic procedure operates in dislodging the substituent, the accompanying calculation fails to give a true picture of the substitution pattern. Because the etherification reaction is usually base catalyzed to generate the starchate ion to which the oxirane reagent is added, the intermediate product is a secondary alkoxide ion of the ring opened epoxide (Parker & Isaacs, 1959). This secondary alkoxide is a slightly stronger nucleophile than the starchate. If the

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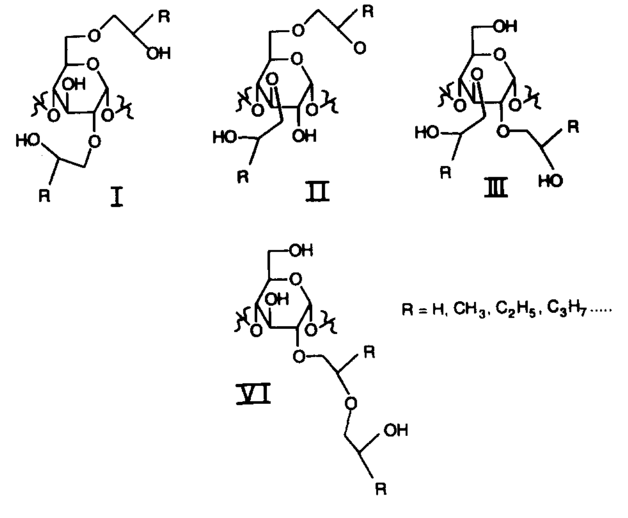


Fig. 1. Pattern of disubstitution of anhydroglucose unit of starch oxirane reagent.

reaction medium is anhydrous a competition ensues between the stronger alkoxide nucleophile and the starchate for incoming epoxide molecules. Thus if two molecules of oxirane are reacted with an anhydroglucose unit, the product distribution barring steric factors would mostly be the 2,2'- with some 2,6- and even 2,3-disubstituted starch ethers (Mercus *et al.*, 1977) in the product mixture (Fig. 1). In the 2,2'-case, the incoming epoxide has been captured by the stronger alkoxide ion on the previously ring opened epoxide, a process described as 'chaining' of the substituent. To adequately represent the complexity of the substitution pattern and hence the properties of the derivative, the term molar substitution (MS), i.e. the number of moles of substituent per anhydroglucose unit (AGU), has been used to describe this substitution pattern (Lee *et al.*, 1983). Although for lower molecular weight starch ethers (2-hydroxyethyl), Lee *et al.* (1983) have improved the chromatographic procedure originally developed by Morgan (Morgan, 1946; Lortz, 1956), the method has not been applied for higher MW hydroxyalky starch ethers because of handling problems involving gaseous ethylene and detector corrosion arising from halogenated reaction products.

For 2-hydroxypropyl starch ether, however, Jones & Riddick (1954, 1957) and Johnson (1969) have shown that the molar substitution ratio of the 2-hydroxypropyl substituent in starch can be quantitatively determined spectrophotometrically at 595 nm via a putative colored complex of propanal with ninhydrin in concentrated H_2SO_4. The simplicity, low cost and accuracy of the procedure makes it attractive as a routine procedure for industrial application.

^{1}H-NMR spectroscopic techniques can also be used to estimate the molar substitution ratios of the higher molecular weight 2-hydroxyalkyl starch ethers. The procedure is somewhat akin to the colorimetric approach as it involves hydrolysis of the substituent, albeit under conditions that cause neither dehydration nor oxidation of the hydroxyalky groups. The major complaint from the ^{1}H-NMR spectroscopist was the lack of availability of standards with which to compare his results. An independent method by which to check the accuracy of the ^{1}H-NMR results was therefore needed; one that could be routinely used at low cost. Such a procedure would enhance analysis of higher MW 2-hydroxyalkyl starch ethers (2-hydroxybutyl and higher C_4-C_{16}) for the authors' plastics work. This paper reports on a modification of this colorimetric technique for the estimation of the MS of 2-hydroxybutyl and *O*-butyl starch ethers, especially as a reliable complementary technique for routine analysis of higher MW hydroxyalkyl starch ethers.

EXPERIMENTAL

General

In-house synthesized *n*-butyraldehyde, and *n*-valeraldehyde were obtained from l-butanol and l-pentanol, respectively, by chromic acid oxidation. Characterization of these aldehydes was done using respective boiling points and FTIR spectrophotometry (Analect RFX-75). C-13 characterization of the sparingly soluble 2-hydroxybutyl and *O*-butyl starch ethers (2-HBS and OBS) was done on a Bruker MSL 300 solid-state NMR spectrometer. Absorbance measurements were performed on a Bausch & Lomb Spectronic 2000 spectrophotometer. Unless otherwise stated, absorbance readings were taken at 30–60 min intervals.

Standards

Two calibration standards, one from an in-house synthesized *n*-butyraldehyde and the other from *n*-valeraldehyde, were prepared as described below.

Procedure

Synthesis of 2-hydroxybutyl starch ether

2-HBS was synthesized using a modification of the procedure of Kesler & Hjermstad (1964). Pearl Starch (100·0 g, A. E. Staley Mfg. Co., Decatur, IL) was placed in a dry 1-liter pyrex reaction kettle provided with a mechanical stirrer, reflux condenser and nitrogen inlet; the reactor being clamped in a 45–50°C oil bath. 2-Propanol (130·0 ml), Na_2SO_4 (15·00 g), NaOH (5·86 g, 0·145 mol) dissolved in H_2O (9·0 ml) were added as the reactor contents were stirred. The reaction flask was purged ~15 min after which 1,2-epoxybutane (36·91 g, 0·52 mol, Aldrich Chemical Co., Milwaukee, WI) was added and the nitrogen was cut off. The reaction mixture was stirred for 60 h after which the oil bath was removed and dilute acetic acid (15·0 ml) was stirred in to pH 5. The solid was filtered, washed in sequence with water, 80% EtOH, absolute EtOH and finally with 85% acetone and dried *in vacuo* at 25°C to yield 112 g, 82%.

The product was identified by FTIR absorption of the C–H bands of the alkyl substituent at 2966, 2931 and 2883 cm^{-1} and the upfield ^{13}C-NMR resonances in the cross polarization-magic angle spinning spectrum at 10 ppm for the CH_3 and 25 ppm for the methylenes. The CH_2-O and CHO of the substituent are overlapped at 60 and 72 ppm, respectively, with corresponding resonances of the C-6 and C-2,3,5 of starch (Fig. 2).

The *O*-butyl starch ether (OBS) was prepared by nucleophilic substitution of iodide in 1-iodobutane with starchate ion. The starchate was first generated using dimsyl anion to abstract hydroxyl protons from starch and 1-iodobutane was then added to the starchate ion. The reaction conditions and work-up were the same as for 2-HBS above.

Standard calibration curves of n-*butyraldehyde and* n-*valeraldehyde*

n-Butyraldehyde (0·50 ml, 0·40 l g^{-1}) was pipetted into a 100-ml dry volumetric flask and diluted to mark. This stock solution was serially diluted with water to 0·00, 2·00, 4·01, 6·01, 8·02, 10·02 × 10^{-5} g ml^{-1}, respectively. From each dilution, 1·00 ml was transferred to one of six 10·00-ml volumetric flasks; the solution was chilled and 5·0 ml concentrated H_2SO_4 added. After mixing the solutions were placed in a 25°C bath to equilibrate. When the temperature was 25°C, 0·20 ml of 3% 2,2-dihydroxyindan-1,3-dione (ninhydrin) in 5% bisulfite was carefully added, mixed and allowed 1 h to develop color. At the end of the interval, the solution was diluted to mark with concentrated H_2SO_4 and mixed; final concentrations being 2·00, 4·01, 6·01, 8·02 and 10·02 × 10^{-6} g ml^{-1}, respectively. Absorbance was measured at 470 nm.

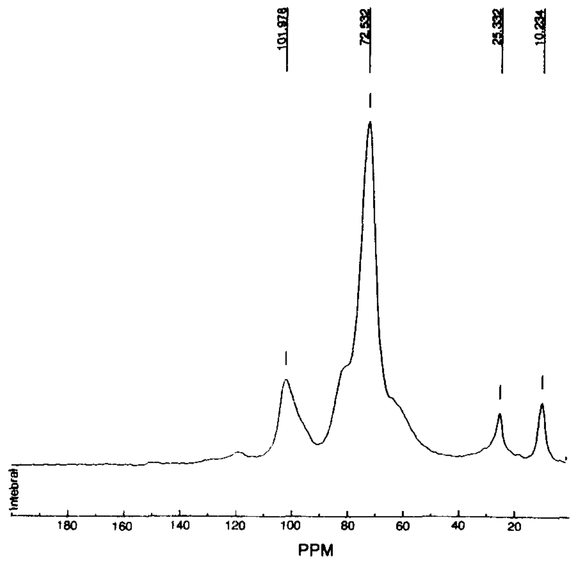


Fig. 2. Solid state ^{13}C-NMR spectrum of 2-hydroxybutyl starch ether.

n-*Valeraldehyde*

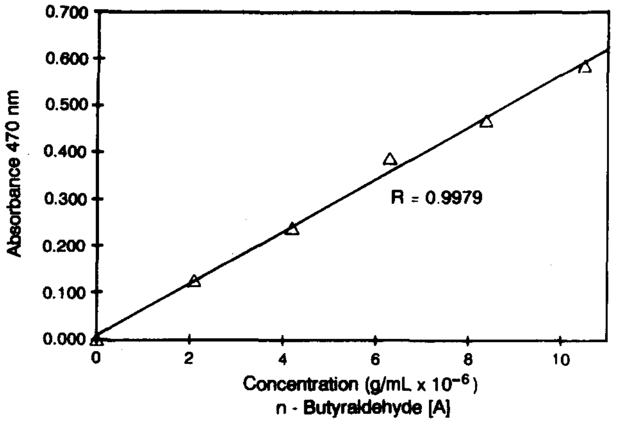
n-Valeraldehyde (0·50 ml, 0·405 g) was serially diluted and treated with 0·60 ml ninhydrin-bisulfite as in the previous section with final concentrations of 0·00, 4·05, 8·10, 16·20, 24·30 and 40·50 × 10^{-6} g ml^{-1}, respectively. Absorbance of colored complex was measured at 547 nm.

Analysis of 2-hydroxybutyl and *O*-butyl starch ethers

Two batches of hydroxybutyl starches (2-HBS$_{18}$ and 2-HBS$_{22}$ synthesized under slightly different experimental conditions; 0·57 mol of 1,2-epoxybutane with a 50-h reaction time instead of 60 h as for 2-HBS$_{18}$) as well as one of OBS$_{28}$ were used. Samples were prepared as described below. 2-*O*-(2-Hydroxybutyl), 2-*O*-butyl starch and unmodified starch (0·0678 g, 0·0483 g and 0·0632 g, respectively) were accurately weighed into three 50·0-ml volumetric flasks. Dilute H_2SO_4 (25·0 ml, 1 N) was added to each sample and placed in a hot water bath until solution was attained. The solutions were cooled to room temperature, diluted to mark with 1 N H_2SO_4 and mixed thoroughly. A 1·0-ml aliquot of sample solution was pipetted into a 25·0-ml volumetric flask, solution chilled and 8·0 ml of concentrated H_2SO_4 was carefully added. The flask was tightly stoppered (glass stopcork), mixed and placed in a water bath maintained at 100°C for 5·0 min. The hydrolyzate was cooled in ice water to 25°C at which point 0·6 ml 3% ninhydrin in 5% $NaHSO_3$ was layered on the solution and mixed. The solution was allowed to develop the colored complex for 1 h at RT as in the previous section. At the end of this period each solution was diluted to mark with concentrated H_2SO_4 and allowed a further 5 min of color development. Absorbance was measured as per *n*-butyraldehyde standard, the starch sample being used as blank in the spectrophotometric run. This procedure was repeated with 0·0709 g, 0·0604 g and 0·0786 g, respectively, of 2-HBS$_{18}$, OBS$_{28}$ and underivatized starch. The 2-HBS$_{22}$ was then done in two replications using 6·65 × 10^{-5} g ml^{-1} with 5·98 × 10^{-5} g ml^{-1} of starch as a control.

RESULTS

Propanal–ninhydrin–bisulfite complex in concentrated sulfuric acid is violet-colored with an absorbance maximum at 595 nm. The intensity of the absorbance for this complex began to gradually decrease after about 20 min at the maximum value (data not shown). *n*-Butyraldehyde and *n*-valeraldehyde on the other hand form red-colored complexes with ninhydrin–bisulfite in concentrated H_2SO_4. The absorbance maxima for these complexes are observed at 470 nm for



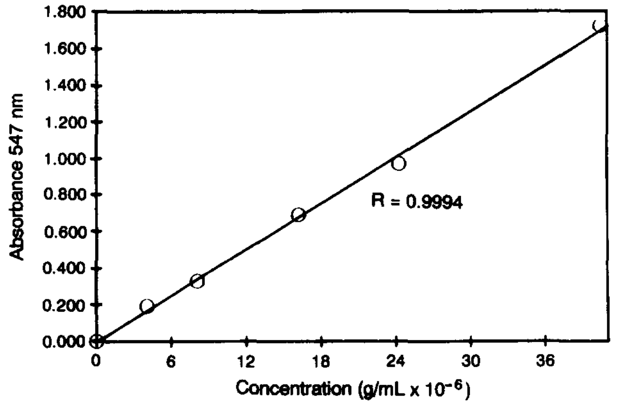


Fig. 3. Standard absorbance-concentration curves for *n*-butyraldehyde- and *n*-valeraldehyde-ninhydrin-bisulfite complexes in concentrated sulfuric acid: (a) *n*-butyraldehyde (in-house); (b) *n*-valeraldehyde (in-house).

the *n*-butyraldehyde complex and 547 nm for the *n*-valeraldehyde complex. Both complexes are stable over several hours and their absorbances can therefore be conveniently measured.

The data obtained for the standards gave linear calibration curves (absorbance-concentration) that obey the Beer-Lambert Law with a correlation coefficient of 0·997 for *n*-butyraldehyde (Fig. 3(a)). For the purpose of applying this procedure to longer chain 2-hydroxyalkyl starch ethers the authors have included the standard calibration plot for *n*-valeraldehyde, correlation coefficient, 0·999 (Fig. 3(b)). The wavelength optimum for the *n*-valeraldehyde complex with ninhydrin-bisulfite in concentrated H_2SO_4 was determined by a scan of the sample in the range 600–400 nm which gave a maximum at 547 nm, the peak intensity reaching a maximum in approximately 4 h, and then dropping off gradually.

To quantify the amount of 2-hydroxybutyl group in 2-hydroxybutyl starch ether the authors utilized the hydrolytic procedure of Johnson (1969), in which a known quantity of substituted starch ether was treated with concentrated sulfuric acid to give a hydrolyzate containing the aldehyde moiety of the substituent, that is, *n*-butyraldehyde in this case (Fig. 4).

Like the *n*-butyraldehyde-ninhydrin-bisulfite complex of the standard each hydrolyzate (2-HBS_{18} and 2-HBS_{22}) gave a characteristic red complex with a maximum intensity of 0·166 absorbance units observed for 2-HBS_{18} at 470 nm. This value for the $5{\cdot}424 \times 10^{-5}$ g ml^{-1} sample was equivalent to the *n*-butyraldehyde concentration of $2{\cdot}862 \times 10^{-6}$ g ml^{-1} or 5·25% substituent. From this per cent value the MS was determined as shown in the equation below to be 0·125 ± 4% when compared to the ^{1}H-NMR result (Table 1). The second run of 2-HBS_{18} gave a MS value of 0·12 ± 0 so that the average deviation for this batch was ±2%. In the same manner the corresponding MS values for 2-HBS_{22} and OBS_{28} were also derived (Table 1).

$$\text{MS} = \frac{\%\ \text{substituent}}{100 - \%\ \text{substituent}} \times \frac{162{\cdot}14}{\text{MW substituent}}$$

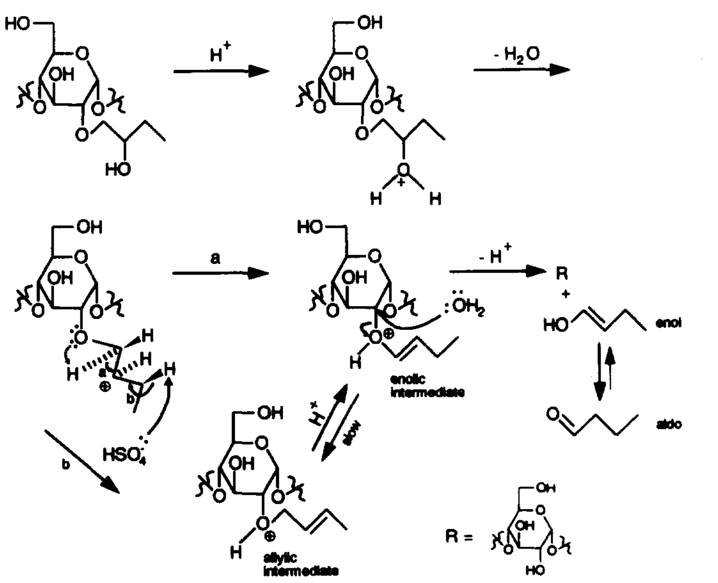


Fig. 4. Proposed mechanism of *n*-butyraldehyde generation from 2-hydroxybutyl starch ether.

where

MS = molar substitution of HBS,
162 = MW of AGU,
substituent = 2-hydroxybutyl group = 72·11.

High-resolution proton NMR spectrometric analysis of hydrolyzates of these samples gave percentage 2-hydroxybutyl and butyl compositions of 4·72%, 3·68% and 1·48%, for the 2-hydroxybutyl starch (2-HBS$_{18}$, 2-HBS$_{22}$) and *O*-butyl starch (OBS$_{28}$) ethers, respectively, The 4·72% value for the 2-hydroxybutyl group in 2-HBS$_{18}$ was reproduced in the colorimetric analysis 2 h before the maximum absorbance intensity was observed (data in parentheses, in Table 1).

DISCUSSION

The maximum intensity of absorbance for both *n*-butyraldehyde and *n*-valeraldehyde complexes with ninhydrin is reached much more slowly than it is for the propanal complex. Whereas the intensity maximum of the latter complex diminished after 20 min, the complexes of *n*-butyraldehyde and *n*-valeraldehyde with ninhydrin–bisulfite in concentrated sulfuric acid have a stability range of 4–22 h at room temperature. Longevity of the complexes is fortunate as it is necessary to obtain a value as close to the maximum intensity as possible for representative data points in the curve fitting process, since the absorbance intensity is concentration-dependent, as shown in the standardization data.

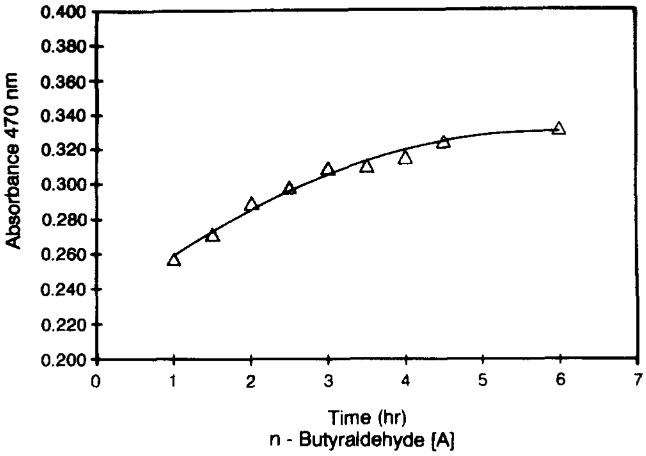
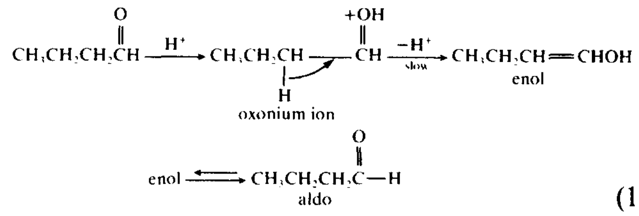


Fig. 5. Time-dependence of absorbance for a single concentration (6·02 × 10^{-6} g ml^{-1}) of the standard *n*-butyraldehyde–ninhydrin–bisulfite complex at 470 nm.

The slower rate of attaining maximum intensity of absorbance may in part be due to the equilibrium nature of the aldehyde–bisulfite reaction, although such an equilibrium condition can be forced to the right by use of excess bisulfite. The other more important factor may be that the aldehyde in the concentrated acid medium exists substantially in the oxonium form which may not contribute to colored complex formation but may slowly rearrange to the enol which then tautomerizes to the strongly complexing aldo form (Fig. 4). In Fig. 5 one observes the increase in absorbance of a single sample of the aldehyde standard with time. A very sharp increase in absorbance during the first hour of measurement is seen, indicating the amount of complexed aldehyde. The change in slope after the first hour is indicative of the slow reconversion of the non-complexing oxonium ion to the aldo form as shown in reaction (1). The available aldo form then leads to complexation, thus driving the enol–aldo equilibrium to the right until all the aldehyde is complexed with ninhydrin–bisulfite and maximum absorbance is attained.



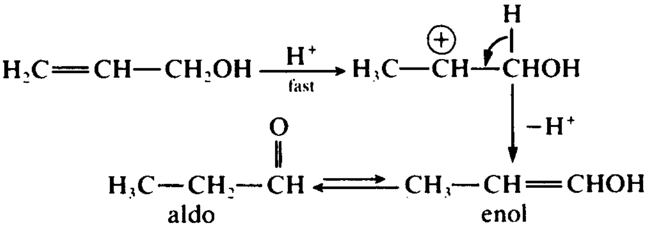
(1)

Table 1. Colorimetric absorbance versus ^{1}H-NMR data of the hydrolyzates of the derivatized starch ethers

Substrate^a	Concentration (g ml^{-1} × 10^{-5})	A_{max} 470 nm	Concentration of substituent × 10^{-6} g ml^{-1}	% Substituent		MS	
				Colorimetric	^{1}H-NMR	^{1}H-NMR	Colorimetric
Starch	5·056	0·000					
2-HBS$_{18}$	5·424	0·166(0·156)	2·862	5·28(4·84)	4·72	0·12	0·125 ± 4%
2-HBS$_{18}$	5·672	0·166	2·162	5·05	4·72	0·12	0·12 ± 0
OBS$_{28}$	3·864	0·052	0·781	2·02	1·48	0·043	0·046 ± 7%
OBS$_{28}$	4·832	0·062	0·938	1·94	1·48	0·043	0·045 ± 5
Starch	6·288	0·000					
2-HBS$_{22}$	6·65	0·132	2·176	3·27	3·68	0·08	0·08 ± 0
2-HBS$_{22}$	6·42	0·114	1·887	2·94	3·68	0·08	0·07 ± 10

^{a}2-HBS$_{18}$ = Replication runs from single batch; 2-HBS$_{22}$ = replication runs from another batch; OBS$_{28}$ = replication runs.

Unlike in the higher homologues (*n*-butyraldehyde and *n*-valeraldehyde), ninhydrin–bisulfite was reported to form a colored complex with both propanal and allyl alcohol (Jones & Riddick, 1957). The latter compound is the other product resulting from the dehydration of 2-hydroxypropyl starch ether and so is naturally present in the product mixture. It is likely that the unsubstituted allyl alcohol in the strong acid medium readily converts to the enol which rapidly tautomerizes to the more stable aldo form that actually results in complex formation; the sulfuric acid simply playing a catalytic role as shown in reaction (2).



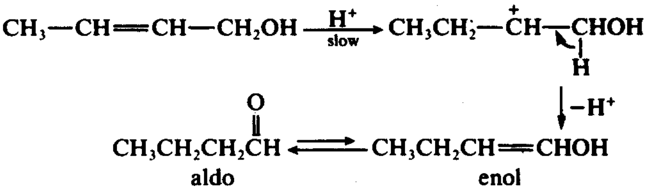
$$H_2C{=}CH{-}CH_2OH \xrightarrow[\text{fast}]{H^+} H_3C{-}\overset{\oplus}{C}H{-}\overset{H}{C}HOH \xrightarrow{-H^+} \underset{\text{enol}}{CH_3{-}CH{=}CHOH} \rightleftharpoons \underset{\text{aldo}}{H_3C{-}CH_2{-}\overset{O}{\overset{\|}{C}}H}$$

(2)

As a consequence of the above interconversion, in the shorter chain allyl alcohol the color intensity reaches a maximum in 1 h.

The dehydration of the 2-hydroxybutyl substituent of the starch ether occurs at the secondary alcoholic function generating a secondary carbonium ion intermediate (Fig. 4). Two pathways are open to this carbocation. In pathway (a) a proton elimination from C-1 of the butyoxy cation results directly in an enolic intermediate which on final hydroxysis tautomerizes rapidly to the aldo form for color reaction. The alternative pathway (b) leads to the formation of the insipient allylic intermediate by proton elimination at C-3 of the butoxy group. Hydrolysis of this last group gives 2-buten-1-ol. This allylic alcohol is slowly protonated probably for steric reasons in contrast to the unhindered allyl alcohol in reaction (2) to generate an α-hydroxy butyl carbocation intermediate. Primary proton loss from C-1 then gives the enol that tautomerizes to the complexing aldo form as in reaction (3). This pathway is mainly responsible for the slower attainment of intensity maximum of the aldehyde-ninhydrin-bisulfite complex.

In conclusion, the close agreement between the ^{1}H-NMR estimate (Dunn, Jr, L. B., unpublished) of the MS of 2-hydroxybutyl and *O*-butyl starch ethers and the results of the spectrophotometric work gives credence to the utility of the procedure for estimating the molar substitution of 2-hydroxybutyl starch ethers. Also the distinct absorbance maximum of the colored complex formed with *n*-valeraldehyde suggests applicability of the method for the determination of the MS of 2-HVS ethers.



$$CH_3{-}CH{=}CH{-}CH_2OH \xrightarrow[\text{slow}]{H^+} CH_3CH_2{-}\overset{+}{C}H{-}\underset{H}{C}HOH \xrightarrow{-H^+} \underset{\text{enol}}{CH_3CH_2CH{=}CHOH} \rightleftharpoons \underset{\text{aldo}}{CH_3CH_2CH_2\overset{O}{\overset{\|}{C}}H}$$

(3)

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