Carbohydrate Polymers **11** (1989) 1–22

Characteristics and Distributions of Ester Groups in Propylene Glycol Alginates

J. F. Kennedy, A. J. Griffiths, K. Philp, D. L. Stevenson, O. Kambanis & C. J. Gray

Department of Chemistry, University of Birmingham, PO Box 363, Birmingham B15 2TT, UK

(Received 22 September 1988; accepted 6 November 1988)

ABSTRACT

The nature of the ester groups in propylene glycol alginate (PGA) has been investigated by ^{13}C-NMR spectroscopy and chemical saponification studies. Two types of ester, namely primary 2-hydroxyprop-1-yl and secondary 1-hydroxyprop-2-yl, were detected by ^{13}C-NMR spectroscopy by virtue of the methyl carbon resonances. From model compound studies the two forms were shown to be due to the type of ester present and not to the type of sugar residue (β-D-mannuronic or α-L-guluronic acid) to which the ester may have been bound. Alkaline saponification studies using a variety of analytical monitoring systems, and model compounds, has shown that the two types of ester exist in an equilibrium with the more thermodynamically favourable primary ester predominating in the ratio 4:1. The nature of the sugar residues, namely β-D-mannuronic and α-L-guluronic acid, to which the esters were bound, did not have any bearing on the rate of ester hydrolysis. The rate of hydrolysis of ester groups in PGA was, however, dependent upon the nature of the ester groups present, namely primary or secondary. On the basis of the evidence presented, the thermodynamically more stable primary ester predominates in PGA, as would be expected from the preparation conditions.

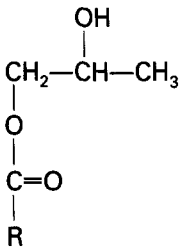
INTRODUCTION

Alginates are algal polysaccharides containing both β-D-mannuronate and α-L-guluronate residues, the proportions of which vary extensively depending upon the source seaweed species, the part of plant the alginate was extracted from, and prevailing climatic conditions at the time of growth. These polysaccharides have numerous applications in both the food and non-food industries (Kennedy *et al.*, 1984; Griffiths &

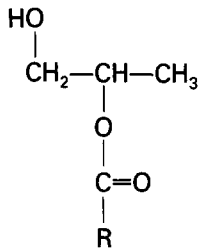
Carbohydrate Polymers 0144-8617/89/$03.50 —

Kennedy, 1988), the specific application usually depending upon the β-D-mannuronate to α-L-guluronate ratio of the polysaccharide used. Of the alginate derivatives, the ester propylene glycol alginate (PGA), prepared from alginic acid by treatment under acid conditions with propylene oxide, is of special commercial importance due to its stability in acidic solutions.

Since propylene oxide is unsymmetrical, two types of ester groups are possible in PGA; these are the primary ester 2-hydroxyprop-1-yl (**I**) and the secondary ester 1-hydroxyprop-2-yl (**II**).



I



II

The nature of the product formed by nucleophilic attack on an unsymmetrical epoxide depends upon the conditions employed. Under basic conditions, attack occurs at the least sterically hindered carbon atom in the ring and yields the primary ester (**I**). Under acidic conditions, however, either ester may be formed depending upon whether the reaction is kinetically or thermodynamically controlled. For instance, Reeve & Sadle (1950) showed that the primary ester is formed predominantly by the action of methanol on propylene oxide in the presence of methoxide, but that a mixture of primary and secondary esters are formed under acidic conditions. Similarly, the acetolysis of styrene oxide (Cohen *et al.*, 1962) yielded a mixture of two esters from which it was concluded that kinetic control gave the secondary ester while conditions leading to the thermodynamically favoured product yielded the primary ester. In another study (Isaacs & Neelakantan, 1968) the acetolysis of propylene oxide led to the formation of primary ester and secondary ester in the ratio of 2:1. While it might be expected that under the conditions used for the preparation of alginate esters (i.e. 60–90°C at acid pH for extended times) the thermodynamically favoured primary ester would predominate, no evidence has been available confirming this or indicating the relative proportions of both types of ester. We now present evidence on these aspects based upon alkaline saponification studies and on NMR spectroscopic data.

MATERIALS AND METHODS

All samples of PGA were provided by Kelco International Ltd, and all chemicals from various suppliers were of analytical grade. Polyhexamethylenebiguanidinium chloride (PHMB) was obtained from ICI Organics Division under the trade name 'Vantocil' 1B.

Synthesis of propylene glycol diacetate

Propylene glycol diacetate was synthesised by refluxing propylene glycol (25 ml) with saturated sodium acetate (10 ml) and acetic anhydride (75 ml) in a 250 ml round-bottom flask for 1·5 h. After refluxing, the mixture was neutralised with sodium bicarbonate and the insoluble oily organic layer was extracted into dichloromethane (5 × 25 ml) which was washed once with water (20 ml) and dried over magnesium sulphate. The product was distilled over the boiling range 185–189°C. GLC analysis (Pye Unicam 104 Chromatograph fitted with 2·5 m × 6 mm i.d. column containing Celite (100 mesh) and a liquid phase of Carbowax (10%), FID detection system) of the product showed the diacetate to be the major product (66%) with considerable traces of both possible monoacetates and propylene glycol making the difference. The diacetate alone was extracted from an aqueous mixture of the components using hexane as solvent.

Synthesis of propylene glycol monoacetate mixture

A mixture of propylene glycol monoacetates were prepared by the acetolysis of propylene oxide. Propylene oxide (10 g) was added to glacial acetic acid (9 ml) and sodium acetate (0·98 g). The mixture was warmed to 50°C until a clear solution was formed (~ 1 h). The liquid was then refluxed for 18 h and left to stand for 2 days whereupon it was filtered to remove sodium acetate. The precipitated sodium acetate was washed with ether and the combined filtrate and ether wash were rotary evaporated to remove both ether and excess propylene oxide. The remaining liquid was treated with water (5 ml) and sodium bicarbonate to form two layers. Thin-layer chromatographic analysis was carried out on silica plates in 3:1 chloroform–hexane mixtures and developed by exposure both to ammonia vapour followed by iodine vapour, and showed the top organic layer to contain a mixture of both monoacetates of propylene glycol.

NMR spectroscopic measurements

The ^{1}H-NMR spectrum of the mixture of propylene glycol monoacetates was recorded on a Perkin Elmer R12B 60 MHz spectrometer at ambient temperature. The mixture was dissolved in carbon tetrachloride and chemical shifts were measured relative to the internal standard tetramethylsilane.

^{1}H-NMR spectra of propylene glycol diacetate dissolved in deuterated chloroform were recorded on a Jeol FX90FT 90 MHz spectrometer at ambient temperature. The width used was 5 μs corresponding to an angle of 30° and four scans were taken at a repetition of 0·1 s. In order to confirm the assignment of the peak at $\delta = 5{\cdot}1$ ppm homonuclear decoupling was used; irradiation of the peak at $\delta = 5{\cdot}1$ ppm caused the multiplet at $\delta = 4{\cdot}1$ ppm to collapse proving that the two are directly linked; likewise irradiation at $\delta = 1{\cdot}15$ ppm caused the peak at $\delta = 5{\cdot}1$ ppm to collapse proving that these two are directly linked. This procedure, together with the integration data, confirmed the structure of propylene glycol diacetate. In all cases chemical shifts were measured relative to tetramethylsilane as reference.

The ^{13}C-NMR spectrum of the propylene glycol monoacetate mixture was recorded on a Jeol FX90FT spectrometer at ambient temperature. The monoacetate concentration was 400 mg ml^{-1} in deuterium oxide, the width was 4 μs corresponding to an angle of 45° and 400 scans were taken at a repetition of 1·5 s. Chemical shifts were measured relative to an internal standard 2,2-dimethyl-2-silapentane-5-sulphonate (DSS).

The ^{13}C-NMR spectrum of dialysed propylene glycol alginate (PGA1) at a concentration of 20% w/v in water was recorded on a Jeol FX90FT spectrometer at ambient temperature. The width used was 12 μs corresponding to an angle of 75° and 40 000 scans were taken at a repetition of 0·2 s. Chemical shifts were relative to DSS. PGA1 was dialysed against running distilled water, freeze-dried and partially hydrolysed at pH 10 for 100 min. The hydrolysate was immediately frozen and freeze-dried. The freeze-dried powder was washed with acetone and dioxan to remove free propylene glycol and freeze-dried again to remove traces of solvent. The ^{13}C-NMR spectrum was recorded at ambient temperature at a width of 12 μs corresponding to an angle of 75° and 41 000 scans were taken at repetition of 0·2 s. Chemical shifts were relative to DSS.

Alkaline saponification studies

Alkaline saponification with polyhexamethylenebiguanidinium chloride (PHMB) monitoring

A 1% (w/v) solution of PGA1 was treated with sodium hydroxide (4M) to a final pH of 10 and maintained at this pH over a period of 5 h with

further additions of sodium hydroxide (1M). At regular intervals aliquots (2·5 ml) of the solution were removed and slowly mixed with PHMB (10 ml, 0·3% w/v) with constant stirring for 5 min. After centrifugation an aliquot (1·0 ml) of the supernatant was removed and diluted to 100 ml with distilled water and the absorbance recorded at 235 nm. When consecutive readings tended to a constant value the pH of the solution was raised to pH 12. Further aliquots (2·5 ml) were once again removed until absorbances tended to a constant value. Since PGA partially precipitates PHMB, the procedure was repeated using distilled water as a control.

Alkaline saponification with free propylene glycol monitoring
Aliquots (4·0 ml) of solutions (1% w/v) of various PGA samples at pH 6·0 were adjusted to pH 10·0 with sodium hydroxide (0·1M) from an autotitrator (Radiometer TT2 Titrator with ABU12 Autoburette) with vigorous stirring. The solution was maintained at pH 10 over a period of hours with further additions from the autotitrator. During this period aliquots (0·1 ml) were withdrawn, added to sodium acetate buffer (1·0 ml, 0·05M, pH 6) and analysed for propylene glycol (see below). When readings tended to a constant value the pH of the solution was raised to pH 12 for 1 h and then analysed for propylene glycol.

Free propylene glycol was assayed using a development of a method originally used for 6-deoxy sugars which contain a free 1-methyl-1,2-diol system (Jones & Somers, 1974) (see Discussion). Sample solutions (100 μl) containing 0–50 μg ml^{-1} of propylene glycol were pipetted into stoppered test tubes and a solution of periodic acid (25 mM) in sulphuric acid (62·5mM, 100 μl) added. The solutions were maintained at ambient temperature for 30 min whereupon sodium metabisulphite (1M, 50 μl) was added. After being allowed to stand for 5 min D-fructose (0·4 mM, 250 μl) was added, followed by a resorcinol reagent (3·0 ml) prepared immediately before use by addition of analytical grade hydrochloric acid (10 vol) to an aqueous stock solution of resorcinol (12 mM, 1 vol). The solutions were then heated at 80°C for 5 min, cooled and the absorbances recorded at 555 nm. The assay was calibrated with propylene glycol and, as controls, the effects of acetate buffer, sodium alginate and PGA upon the assay were determined. The assay was fully automated using Technician AA1 modules as shown in Fig. 1, whilst the calibration is shown in Fig. 2.

Alkaline saponification of propylene glycol diacetate with HPLC monitoring

A solution of propylene glycol diacetate (4·0 ml, 0·05% w/w) was titrated with sodium hydroxide (0·1M) from an autotitrator to maintain the solu-

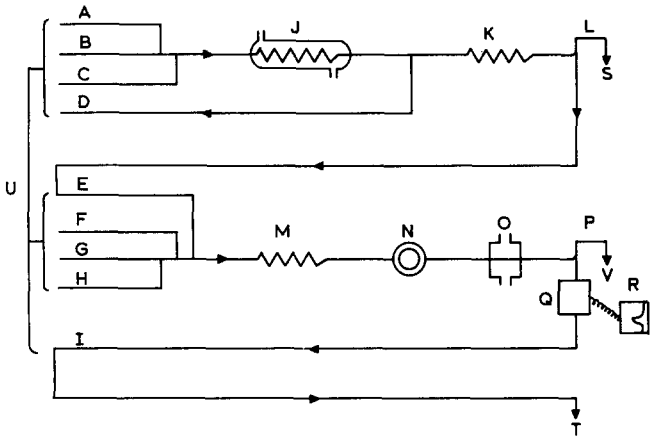


Fig. 1. Automated assay system for the detection of propylene glycol. A, Air, 0·23 ml min^{-1}; B, periodic acid (25mM) in sulphuric acid (62·5mM), 0·1 ml min^{-1}; C, sample, 0·1 ml min^{-1}; D, sodium metabisulphite (1·0M), 0·05 ml min^{-1}; E, return, 0·1 ml min^{-1}; F, D-fructose (0·4mM), 0·05 ml min^{-1}; G, resorcinol–HCl reagent, 0·42 ml min^{-1}; H, air, 0·6 ml min^{-1}; I, return, 0·42 ml min^{-1}; J, reaction heating coil, 30°C, time 20 min; K, cooling coil; L, debubbler; M, mixing coil; N, oil bath, 90°C, time 3 min; O, water-cooled jacket; P, debubbler; Q, colorimeter 550 nm; R, chart recorder; S, to waste, T, to waste; U, pass through peristaltic pump; V, to waste.

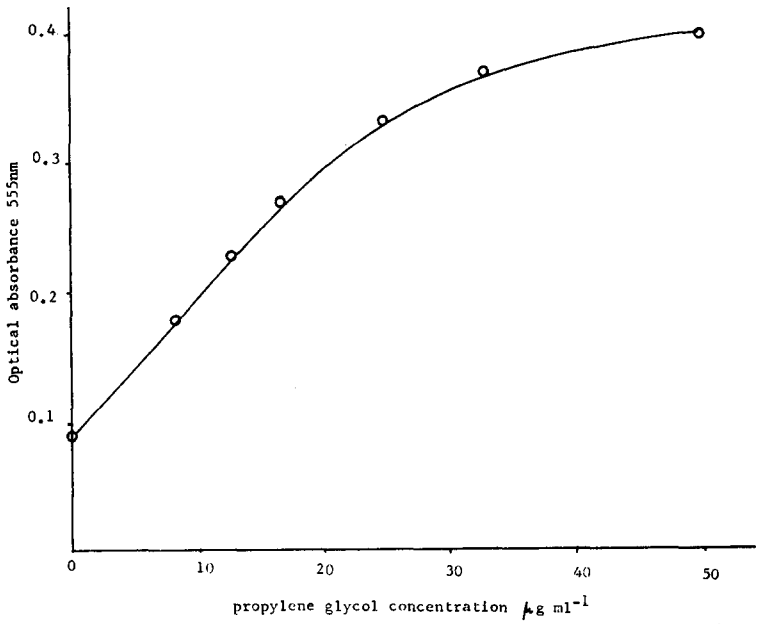


Fig. 2. Calibration curve for the determination of propylene glycol by the D-fructose–resorcinol assay.

tion at pH 10 over a period of 5 h. At intervals aliquots (100 μl) were withdrawn and added to sodium acetate buffer (1·0 ml, 0·05M, pH 6). Aliquots (200 μl) of the buffered samples were analysed by HPLC using a Spherisorb ODS column (25 × 0·45 cm i.d.) fitted to a Constametric III pump and Waters Associates Wisp 710B Autoinjector. Detection was performed at 220 nm using a Spectromonitor III UV detector coupled to a Linseis chart recorder. The column was eluted with acetonitrile solution (5% v/v, 1·5 ml min^{-1}) and the column was washed with methanol (80% v/v) for 10 min prior to each loading. Using this system only the diacetate and two monoacetates of propylene glycol were detected since propylene glycol does not absorb at 220 nm. Propylene glycol concentrations were therefore determined (as a percentage of the total) by difference using the detected values of the other components within the mixture.

RESULTS

NMR spectroscopic studies

The ^{13}C-NMR spectrum of dialysed PGA1 as a 20% (w/v) solution is shown in Fig. 3. Two resonances were observed in the chemical shift area which normally corresponds to methyl groups, a minor resonance at $\delta = 18{\cdot}3$ ppm and a major resonance at $\delta = 21{\cdot}0$ ppm with relative intensities of 4841% and 18560% respectively. It was theoretically calculated (Williams & Fleming, 1980) that the methyl carbon resonance of the primary ester **I** should appear downfield relative to the methyl carbon resonance of the secondary ester **II**. On this basis, and calculating from relative intensities, the primary ester **I** is present as 79·3% of the total ester content whereas the secondary ester **II** is present as 20·7% of the total ester content.

Since differences in the chemical shifts of the methyl carbon resonances could possibly be due to the nature of the sugar residue the ester is bound to, either β-D-mannuronic acid or α-L-guluronic acid, and not to the type of ester present, unequivocal assignments were made using model compounds. The ^{1}H-NMR spectrum is shown in Fig. 4 for propylene glycol diacetate. From the integration data it is clear that the resonances can be assigned as follows: $\delta = 1{\cdot}15$ ppm are methyl protons ($C\underline{H}_3$) at C-3; $\delta = 1{\cdot}95$ ppm are $C\underline{H}_3$ protons of acetyl groups; $\delta = 4{\cdot}10$ ppm are methylene protons ($-C\underline{H}_2OAc$); and $\delta = 5{\cdot}1$ ppm is the proton on the secondary position ($AcOCH_2-C\underline{H}(OAc)-CH_3$). Homonuclear decoupling experiments confirmed the structure of the diacetate.

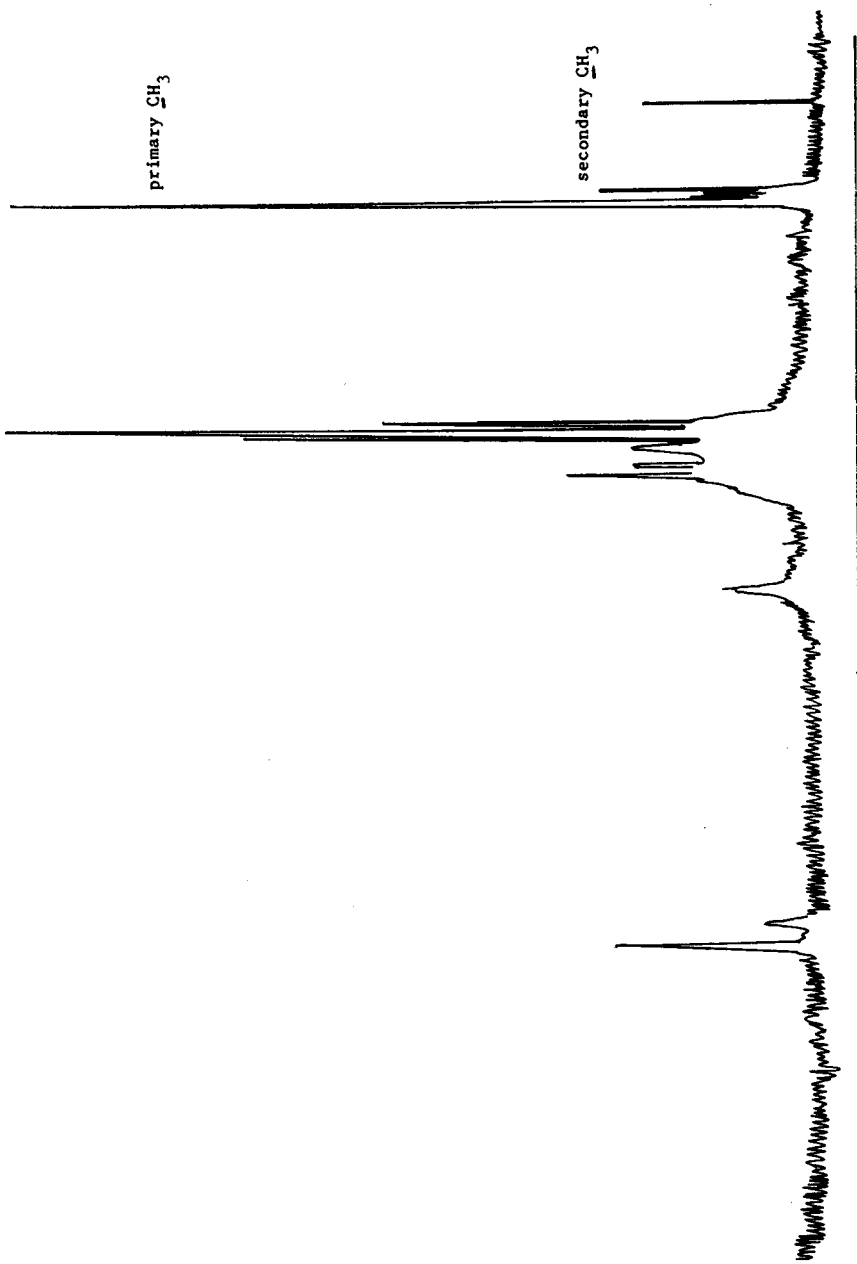


Fig. 3. C^{13}-NMR spectrum of unhydrolysed PGA1.

The ^{1}H-NMR spectrum of the mixture of propylene glycol monoacetates is shown in Fig. 5. It is immediately clear from Fig. 5 that the mixture is that of monoacetates since the methyl proton resonances on C-3 are roughly the same intensity as the methyl protons on the acetyl groups (116 and 121 respectively). The integral of the resonance at $\delta = 4{\cdot}1$ ppm due to the methylene protons bound immediately adjacent to an acetyl group ($C\underline{H}_2$—OAc) was equal to 68 whereas the integral at $\delta = 5{\cdot}1$ due to the proton on the secondary carbon atom to which an acetyl group is bound ($CH_2OAcC\underline{H}(OAc)CH_3$) was 16. Since the methylene group at $\delta = 4{\cdot}1$ ppm contains 2 protons the relative intensity of this group to the secondary proton at $\delta = 5{\cdot}1$ ppm is 68/2:16, or in other words, the ratio of primary to secondary ester is 2·1:1, which is in excellent agreement with data previously reported on the acetolysis of propylene oxide (Isaacs & Neelakantan, 1968).

The ^{13}C spectrum of the propylene glycol monoacetates is shown in Fig. 6. Since ^{1}H-NMR of this mixture revealed it to be a 2:1 mixture of primary:secondary esters then a complete assignment of all resonances in the ^{13}C-NMR spectrum can be made (Table 1), using the intensities of each resonance. Since the chemical shifts of the methyl carbon resonances for both primary and secondary esters (Table 1) correspond to the chemical shifts for the methyl carbon resonances in PGA1 it is clear that differences in the methyl carbon resonances in PGA1 is due to the type of ester present (primary or secondary) and not to differences in sugar residues to which the esters are bound.

Alkaline saponification studies

The results of the alkaline hydrolysis of PGA1 by monitoring with the polyhexamethylenebiguanidinium chloride (PHMB) assay (Kennedy & Bradshaw, 1986, 1987) are shown graphically in Fig. 7. From Fig. 7 the relative amounts of each type of ester present and the total degree of esterification in PGA1 can be calculated as follows (see Fig. 7):

$$\% \text{ primary ester} = \frac{A-B}{A-C} \times 100$$

$$\% \text{ secondary ester} = \frac{B-C}{A-C} \times 100$$

$$\% \text{ of esterification} = \frac{A-C}{I-C} \times 100$$

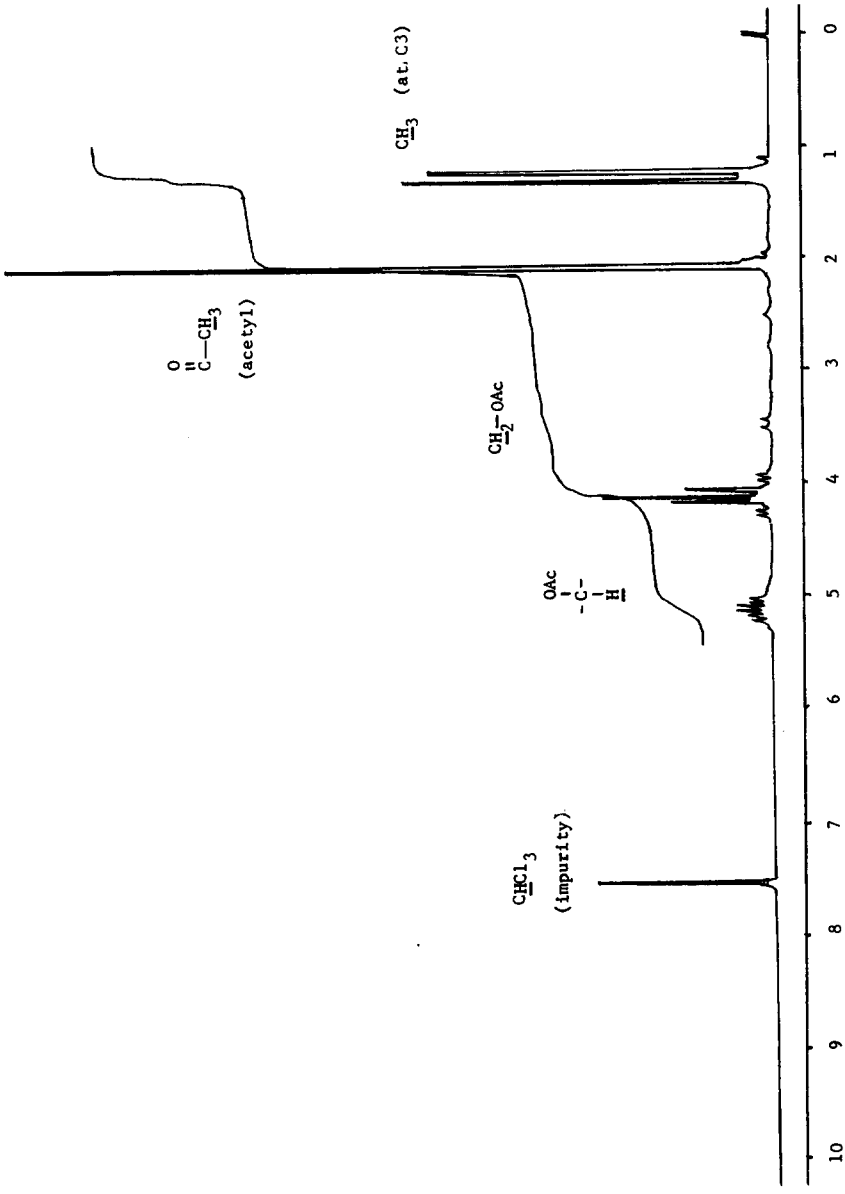


Fig. 4. ^{1}H-NMR spectrum of propylene glycol diacetate.

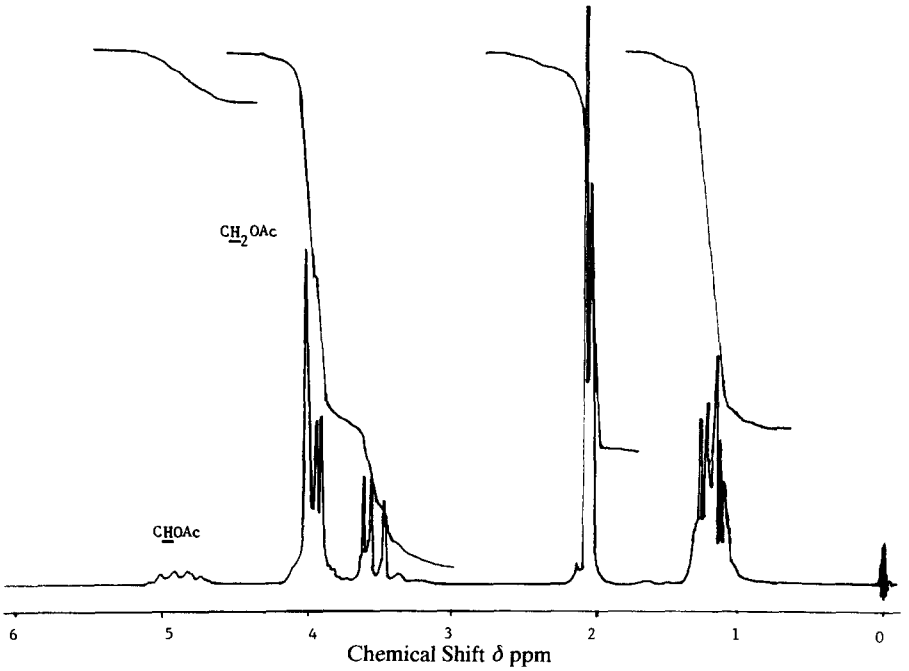


Fig. 5. ^{1}H-NMR spectrum of a mixture of propylene glycol monoacetates.

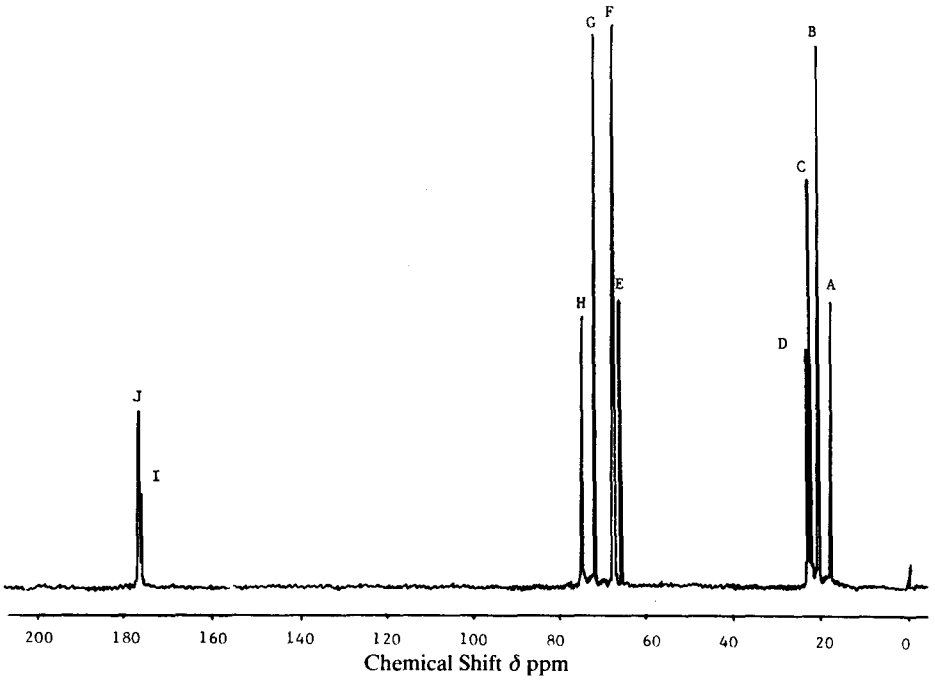


Fig. 6. ^{13}C-NMR spectrum of a mixture of propylene glycol monoacetates (see also Table 1).

TABLE 1
Complete ^{13}C-NMR Assignment of All Resonances in the Mixture of Propylene Glycol Monoacetates

Chemical shift[a] *(ppm)*	*Intensity*[b] *(%)*	*Assignment*	
18·187	3646 (A)	$\underline{C}H_3-CH-OAc$	II
21·081	6799 (B)	$\underline{C}H_3-CH-OH$	I
23·130	5133 (C)	$CH_2-O-\overset{\overset{O}{\|\|}}{C}-\underline{C}H_3$	I
23·585	3018 (D)	$CH_3-\underset{\underset{CH_2OH}{\|}}{CH}-O-\overset{\overset{O}{\|\|}}{C}-\underline{C}H_3$	II
66·764	3682 (E)	$-\underline{C}HOAc$	II
67·967	7070 (F)	$-\underline{C}H_2-OAc$	I
72·129	6955 (G)	$-\underline{C}H-OH$	I
75·087	3455 (H)	$-\underline{C}H_2-OH$	II
176·272	1228 (I)	$CH_3-\underset{\underset{CH_2OH}{\|}}{CH}-O-\overset{\overset{O}{\|\|}}{\underline{C}}-CH_3$	II
176·532	2297 (J)	$CH_3-\underset{\underset{OH}{\|}}{CH}-CH_2O-\overset{\overset{O}{\|\|}}{\underline{C}}-CH_3$	I

[a] Relative to internal standard 2,2-dimethyl-2-silapentane-5-sulphonate.
[b] Letters in brackets denote peaks, see Fig. 6.

where $A = OD^{235}$ of PGA1 before hydrolysis; $B = OD^{235}$ of PGA1 after hydrolysis at pH 10; $C = OD^{235}$ of PGA1 after hydrolysis at pH 12; $I = OD^{235}$ of control (i.e. no PGA1 added to PHMB).

From this analysis the percentage of primary ester was found to be 76% whilst the secondary ester content was 24%. The total degree of esterification in PGA1 was 82% corresponding to 18% free carboxylate groups in the polymer. This analysis for the relative content of each type of ester present is in excellent agreement with ^{13}C-NMR data (primary ester 79·3%, secondary ester 20·7%).

Since PHMB is not specific for alginic acid and will be precipitated by any anionic polymeric species, the rate of hydrolysis of PGA was monitored by assaying for free released propylene glycol, by a specific

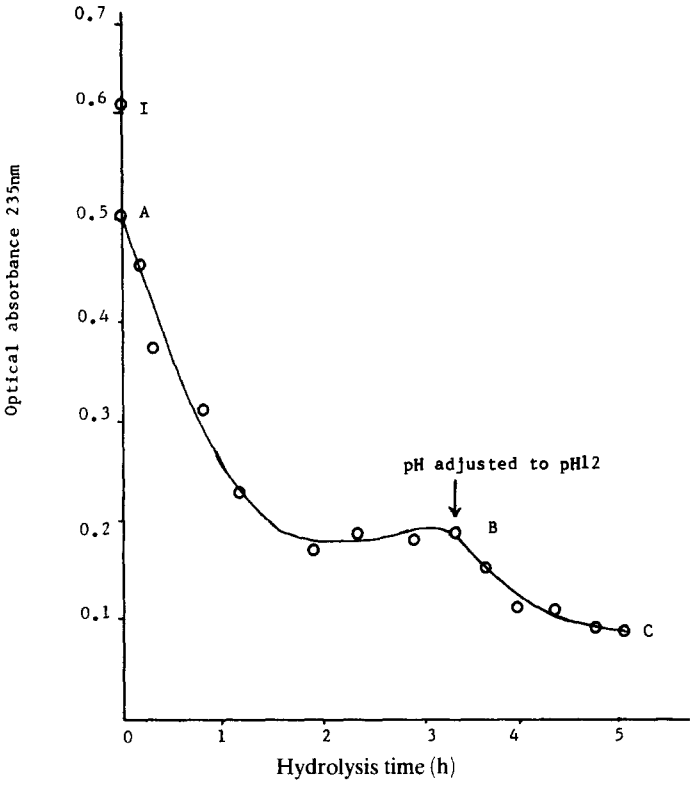


Fig. 7. Hydrolysis of PGA1 with PHMB assay monitoring.

TABLE 2
Degree of Esterification and Extent of Alkaline Hydrolysis for 2-Year-Old Propylene Glycol Alginate Samples

Sample reference number	*Degree of esterification (%)*	*Fraction of ester hydrolysed at pH 10 (%)*
PGA1	82	75
PGA2	14	72
PGA3	37	68
PGA4	43	65
PGA5	66	73
PGA6	48	69

assay, which was found to be free from interference from PGA, sodium alginate and acetate buffer, linear within the range 0–30 μg ml^{-1} of propylene glycol and could be easily automated. By using this assay a series of PGA samples PGA1 and PGA2–6 of varying degrees of

TABLE 3
Degree of Esterification and Extent of Alkaline Hydrolysis of Freshly Prepared Propylene Glycol Alginate Samples

Sample reference number	*Sample reaction time (h)*[a]	*Degree of esterification (%)*	*Fraction of ester hydrolysed after 4 h at pH 10 (%)*
PGA7	1	40	63
PGA8	2·25	58	67
PGA9	4	63	73
PGA10	5	71	72
PGA11	6·25	69	72

[a] Exposure of alginate to propylene oxide.

TABLE 4
Degree of Esterification and Extent of Alkaline Hydrolysis of Propylene Glycol Alginates from Different Seaweed Sources

Sample reference number	*Source of alginate*	*Degree of esterification (%)*	*Fraction of ester hydrolysed after 4 h at pH 10 (%)*	*M:G ratio*[a]
PGA12	*Ascophyllum nodosum*	68	70	High *M*
PGA13	*Ascophyllum nodosum* and *Lessonia flavicans*	72	69	Intermediate
PGA14	*Lessonia nigrescens*	65	68	High *G*

[a] High *M* is β-D-mannuronic to α-L-guluronic acid ratio above ~ 53:47; High *G* is β-D-mannuronic to α-L-guluronic acid ratio of ~ 30:70; Intermediate is β-D-mannuronic to α-L-guluronic acid ratio of ~ 50:50.

esterification prepared 2 years previously, PGA samples PGA7–11 of varying degrees of esterification prepared freshly, and a series of PGA samples PGA12–14 prepared from various alginate sources of differing β-D-mannuronic acid to α-L-guluronic acid ratios were analysed. The final results are shown in Tables 2, 3 and 4, respectively, whereas the reaction profiles against time are shown in Figs 8, 9 and 10, respectively.

In order to verify that only primary ester groups are hydrolysed at pH 10, propylene glycol diacetate was hydrolysed at pH 10 over a period of 5 h and the reaction products analysed periodically by HPLC. The reaction profile is shown in Fig. 11. Since hydrolysis of propylene glycol diacetate at pH 10 was expected to yield only the secondary ester it is

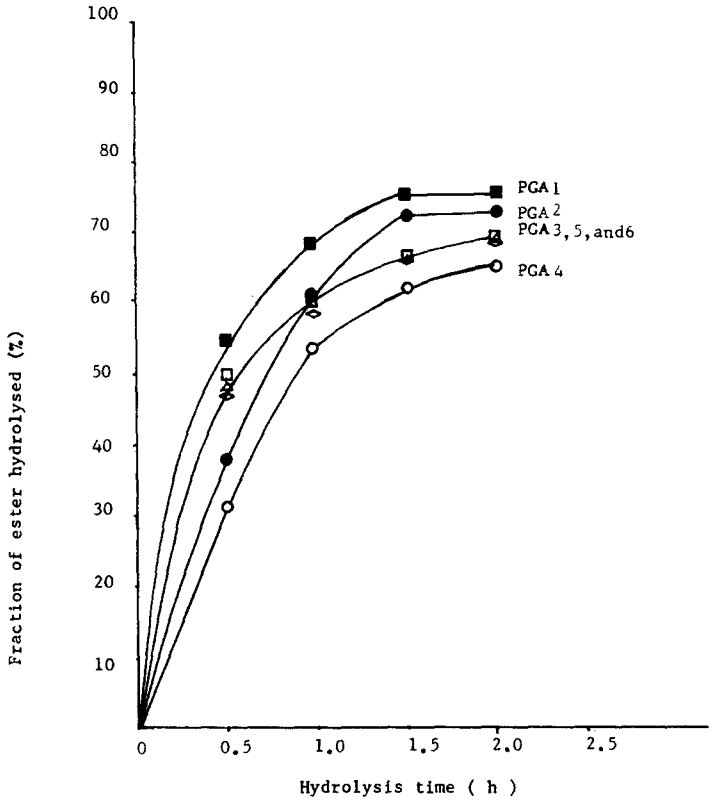


Fig. 8. Reaction profile of the alkaline hydrolysis of 2-year-old PGA samples with sodium hydroxide (0·1M) at pH 10 and 25°C. ■, PGA1; ●, PGA2; ◇, PGA3; ○, PGA4; △, PGA5; □, PGA6.

clear from Fig. 11 that the mechanism of hydrolysis was not as simple as expected since both propylene glycol monoacetates and the final hydrolysis product propylene glycol were all detected at an early stage into the hydrolysis.

Since the hydrolysis mechanism for the model compound propylene glycol diacetate was not as expected PGA1 was hydrolysed at pH 10, immediately frozen and freeze-dried, washed with acetone and dioxane to remove residual propylene glycol and the ^{13}C-NMR spectrum recorded (Fig. 12). It is clear from Fig. 12 that both primary and secondary esters are still present and not secondary ester only. From the integrals it was calculated that this hydrolysed PGA1 contained 85·5% primary ester groups and 14·5% secondary ester groups of the total ester content.

Comparing intensities of ester resonances of partially hydrolysed PGA1 to unhydrolysed PGA1 and correcting for solution concentra-

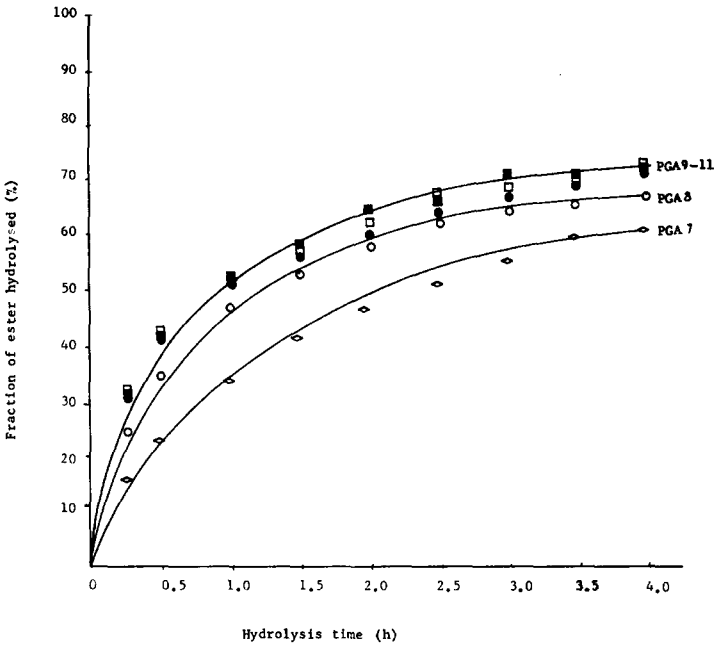


Fig. 9. Reaction profile of the alkaline hydrolysis of freshly prepared PGA samples with sodium hydroxide (0·1M) at pH 10 and 30°C. ◇, PGA7; ○, PGA8; ●, PGA9; ■, PGA10; □, PGA11.

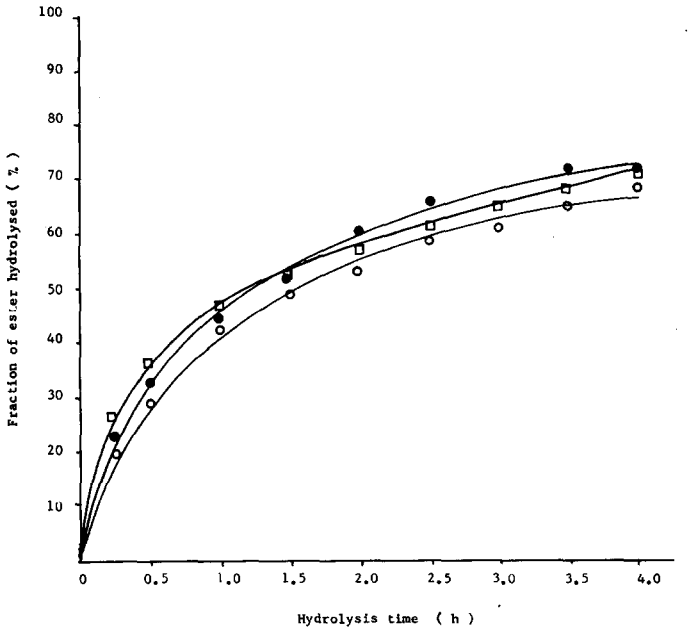


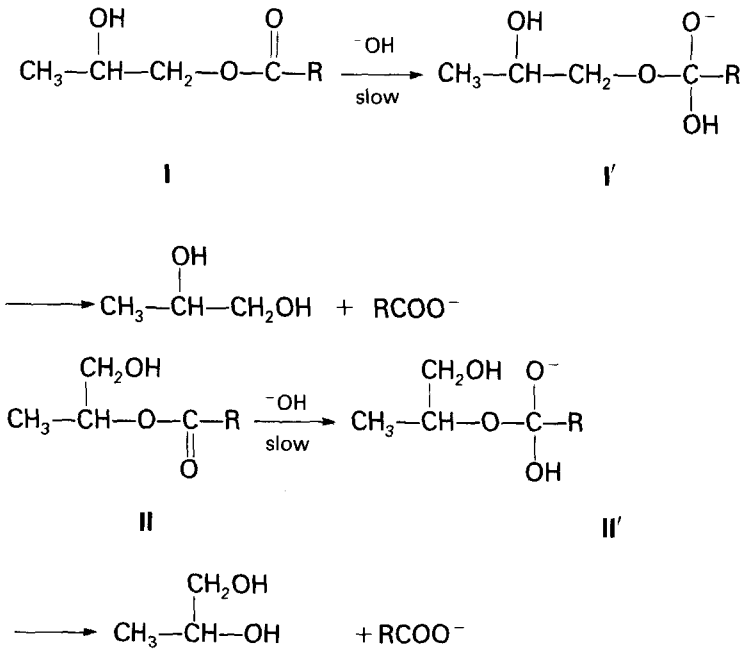
Fig. 10. Reaction profile of the alkaline hydrolysis of PGA samples of varying β-D-mannuronic acid to α-L-guluronic acid ratios with sodium hydroxide (0·1M) at pH 10 and 30°C. ●, PGA12; ○, PGA13; □, PGA14.

tions then 51·7% of the ester groups were hydrolysed at pH 10 over a period of 100 min.

DISCUSSION

The ^{13}C-NMR spectrum of PGA1 (Fig. 3) clearly shows the presence of two different carbon methyl resonances at $\delta = 18{\cdot}3$ and 21·0 ppm. Synthesis of model compounds and analysis by both ^{1}H and ^{13}C-NMR has shown unequivocally that the resonances are due to two different types of ester groups, namely primary ester and secondary ester and not due to the two possible sugar residues to which the ester may be bound namely β-D-mannuronic acid or α-L-guluronic acid. From the intensity of the resonances it is clear that the primary ester is present as the major component (79·3%) whereas the secondary ester is present as the minor component (20·7%). On this basis it is clear that the thermodynamically favoured product predominates as expected.

Further evidence for the existence of two types of esters in PGA was obtained from alkaline saponification studies. The mechanism of ester saponification is well known (March, 1977) and proceeds in the following manner;



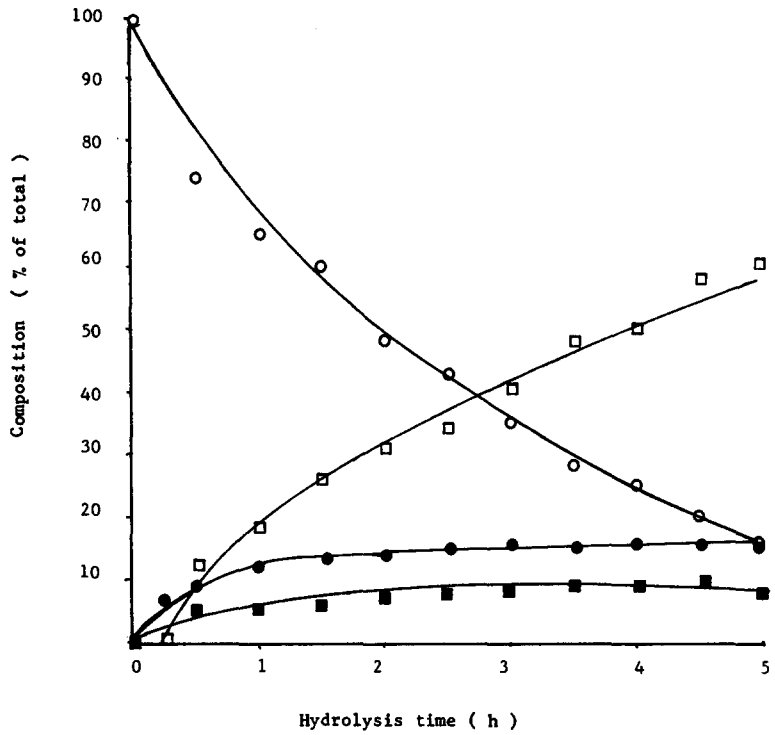


Fig. 11. Reaction profile of the alkaline hydrolysis of propylene glycol diacetate with sodium hydroxide (0·1M) at pH 10 and 30°C. ○, Propylene glycol diacetate; ●, propylene glycol monoacetate (primary); ■, propylene glycol monoacetate (secondary); □, propylene glycol.

Since this reaction proceeds via such a pathway the more sterically crowded intermediate **II'** is expected to be saponified at a much lower rate than the less sterically crowded intermediate **I'**. Therefore, at pH 10 the primary ester **I** can be expected to be preferentially hydrolysed relative to the secondary ester **II**. A differential titration should therefore give the relative proportion of each type of ester in PGA.

Hydrolysis of PGA1 and monitoring the hydrolysis by the PHMB assay (Kennedy & Bradshaw, 1986, 1987) produced a reaction profile (Fig. 7) from which it was calculated that the primary ester was present as 76% of the total ester content while the secondary ester was present as 24% of the total ester content in excellent agreement with ^{13}C-NMR data. However, as shown in Fig. 7 there is a rapid initial hydrolysis of ester followed by a period of slower hydrolysis which never quite levels off. Since the basis of the PHMB assay is the precipitation of PHMB by any polymeric anionic species, thereby removing it from solution and

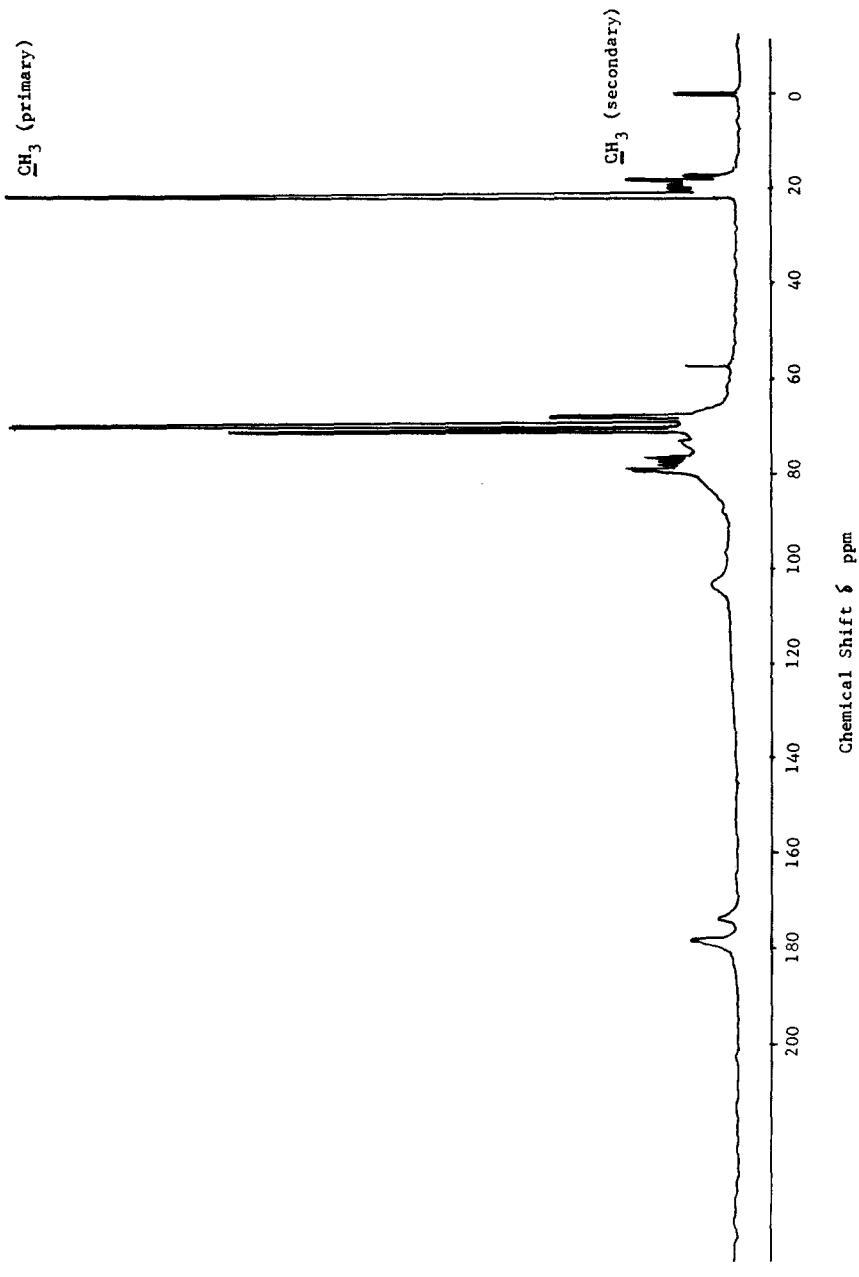


Fig. 12. ^{13}C-NMR spectrum of hydrolysed PGA1.

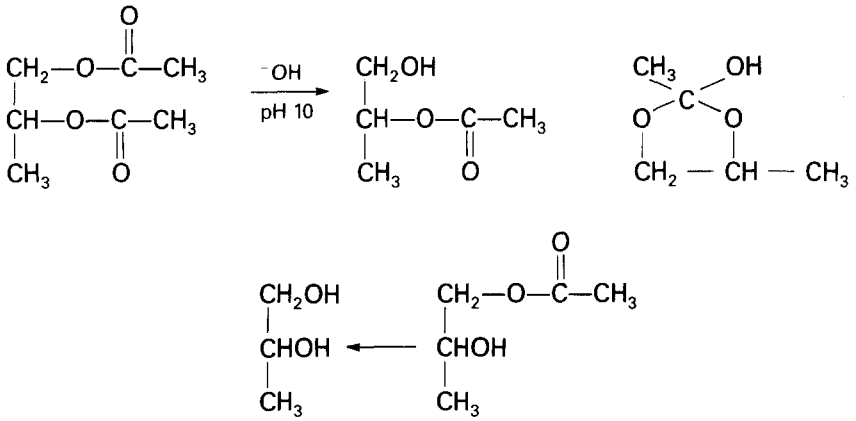
determining the residual PHMB by UV spectrophotometry, the assay is not specific and this non-levelling out of the curve could be due to interference. Nevertheless, a further rapid hydrolysis is seen when the pH is raised to pH 12 to complete the reaction.

In order to monitor the hydrolysis of PGAs by a specific assay a new colorimetric assay was developed. Periodate oxidation of free propylene glycol gives acetaldehyde which causes interference in the determination of D-fructose with resorcinol (Roe, 1934) by causing a shift in wavelength of the absorption chromophore from 480 to 555 nm with enhancement of absorption. The assay is linear over the range 0–30 μg ml^{-1} of propylene glycol (Fig. 2), free from interference by PGA, sodium alginate and acetate buffer, and easily automated (Fig. 1).

Hydrolysis of PGA1 and 2-year-old PGA samples PGA2–6 of varying degrees of esterification, at pH 10 showed that the primary to secondary ester ratio was roughly 70 to 30 in all cases (Table 2), with specific monitoring for propylene glycol by the new colorimetric assay. However, once again as can be seen in the reaction profiles (Fig. 8) there is an initial rapid release of propylene glycol which does not level off after 2 h. Similarly, the ratio of primary to secondary esters in freshly prepared PGA samples PGA7–11 of varying degrees of esterification was 70 to 30. However, as can be seen from the reaction profile (Fig. 9) the rate of release of propylene glycol from these freshly prepared PGAs was much slower than for the 2-year-old samples (Fig. 8), and once again this never quite levels out. When PGA samples PGA12–14 of differing β-D-mannuronic acid to α-L-guluronic acid ratios but with similar degrees of esterification were assayed by the same method, once again the primary ester to secondary ester ratio was 70 to 30 (Table 4). The reaction profile (Fig. 10) for these samples was similar to that of the freshly prepared samples PGA7–11 (Fig. 9). The rate of release of propylene glycol from PGA is therefore independent of β-D-mannuronic acid to α-L-guluronic acid ratios. The major effect relating to the release of propylene glycol from PGA on hydrolysis at pH 10 therefore remained unsolved.

In order to obtain information about the mechanism of hydrolysis of PGA at pH 10 the model compound propylene glycol diacetate was hydrolysed at pH 10 and the hydrolysis products monitored by HPLC (Fig. 11). It is immediately obvious from Fig. 11 that there is a very rapid decrease in propylene glycol diacetate concentration with a constant ratio throughout of 2:1 for primary propylene glycol monoacetate to secondary propylene glycol monoacetate. This result was totally unexpected since hydrolysis of propylene glycol diacetate at pH 10 should have produced secondary propylene glycol monoacetate only, by

virtue of removing the one primary acetate ester group. In order for the reaction to proceed further at this pH the secondary propylene glycol monoacetate must have been converted into primary propylene glycol monoacetate which was then hydrolysed to propylene glycol. The constant ratio of both propylene glycol monoacetates throughout the course of the reaction would support such an equilibrium system. The mechanism for the hydrolysis of propylene glycol diacetate is therefore likely to involve hydrolysis at the primary ester position giving the secondary monoester and conversion of this secondary monoester into the primary monoester, probably via the intermediate cyclic *ortho* ester form (Cohen *et al.*, 1962).



Partial hydrolysis of PGA1 at pH 10 with immediate freezing and re-running the ^{13}C-NMR spectrum (Fig. 12) showed the presence of both primary and secondary esters in the ratio 85·5:14·5. Clearly the hydrolysis of PGA1 also involves an equilibrium between both types of ester since the secondary ester was expected to be the predominant component. PGA1 therefore exists as an equilibrium mixture containing both primary and secondary ester in which the more thermodynamically favourable primary ester predominates. The initial hydrolysis of PGA at pH 10 proceeds rapidly (Figs 7–10), whereby primary ester groups are removed, and as the hydrolysis proceeds a rapid decrease in the rate of saponification is observed. During this phase in the hydrolysis an equilibrium system between primary and secondary ester groups is established with the primary ester predominating. Given enough time therefore, PGA will be completely hydrolysed at pH 10 since secondary ester groups interconvert into primary ester groups since the equilibrium is strongly in favour of the more thermodynamically stable primary ester. From ^{13}C-NMR data on untreated PGA, the ratio of primary to secondary ester groups in the system is 4:1.

CONCLUSION

Both ^{13}C-NMR spectroscopy and chemical saponification studies have established that there are two types of ester groups in PGA, which are in an equilibrium system with the more thermodynamically favourable primary 2-hydroxyprop-1-yl ester predominating over the secondary 1-hydroxyprop-2-yl ester in a ratio of 4:1. It has been shown that the secondary ester readily converts to primary ester suggesting that during the preparation of PGA under acidic conditions the secondary ester is initially produced under kinetic control which in turn converts under thermodynamic control to primary ester with primary ester predominating in a ratio of 4:1. The secondary ester groups were found to be more resistant to alkaline hydrolysis than the primary ester groups and the nature of the sugar residue to which the esters were bound had no bearing on the rate of ester hydrolysis.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support and provision of all propylene glycol alginate samples from Kelco International Ltd, Girvan, Scotland, and an SERC CASE award (A.J.G.).

REFERENCES

Cohen, T., Dughi, M., Notaro, A. & Pinkhus, G. (1962). *J. Org. Chem.*, **27**, 814.
Griffiths, A. J. & Kennedy, J. F. (1988). In *Carbohydrate Chemistry,* ed. J. F. Kennedy. Oxford University Press, Oxford, pp. 597–635.
Isaacs, N. S. & Neelakantan, K. (1968). *Can. J. Chem.*, **46**, 1043.
Jones, R. G. & Somers, P. J. (1974). *Carbohydr. Res.*, **42**, 162.
Kennedy, J. F. & Bradshaw, I. J. (1986). *Carbohydr. Res.*, **156**, 79.
Kennedy, J. F. & Bradshaw, I. J. (1987). *Carbohydr. Polym.*, **7**, 35.
Kennedy, J. F., Griffiths, A. J. & Atkins, D. P. (1984). *Gums and Stabilizers for the Food Industry,* Vol. 2. eds G. O. Phillips, D. A. Wedlock & P. D. Williams. Pergamon Press, Oxford, p. 417.
March, J. (ed.) (1977). In *Advanced Organic Chemistry* (2nd edn). McGraw-Hill, Tokyo.
Reeve, W. & Sadle, A. (1950). *J. Am. Chem. Soc.*, **72**, 1251.
Roe, J. H. (1934). *J. Biol. Chem.*, **107**, 15.
Williams, D. H. & Fleming, I. (1980). In *Spectroscopic Methods in Organic Chemistry.* McGraw-Hill, London.