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Cellulose derivatives with low DS. II. Analysis of alkanoates

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The determination of the degree of substitution (DS) of cellulose alkanoates representing a wide range of ester types (acetate (C-2), to stearate (C-18), with a DS of 0 to 3) was performed by ^{1}H-NMR spectroscopy and aminolysis/gas chromatography. Poor solubility combined with complex substituent signals preclude proton NMR spectroscopy as a method of analysis when the DS of alkanoates falls below one. This is true for all ester-derivative types, especially for the derivatives with bulky substituent groups. By contrast, the aminolysis/gas chromatography method is independent of both solubility and DS, and thus represents the method of choice for cellulose esters which have low degrees of substitution and bulky substituent groups..

INTRODUCTION

Cellulose is the world's most abundant linear polymer. Its isolation and purification (by the pulp and paper industry) represents one of the largest industries worldwide. Yet, cellulose is hardly ever used as a *chemical raw material* because the abundant hydrogen bonding of its anhydroglucose repeat units (intra- and intermolecularly) prevents cellulose from being processable by normal melt (or solution) technologies. The resolution of hydrogen bonds in cellulose by heating requires temperatures that exceed its thermal decomposition temperature.

This processing handicap is conventionally overcome by chemical modification of cellulose (i.e. cellulose derivatization). This is normally accomplished heterogeneously, by esterification or etherification. Although several novel solvent systems have recently created opportunities for the homogeneous synthesis of cellulose derivatives (McCormick & Dawsey, 1990; Isogai *et al.*, 1986), in industry, cellulose derivatives are produced by heterogeneous synthesis, and the reactions are driven to high degrees of substitution (DS) because the partial crystallinity of cellulose otherwise results in extensive non-uniform substitution along the cellulose backbone. Cellulose alkanoates with a high DS are usually analyzed by ^{1}H-NMR spectroscopy (Buchanan *et al.*, 1987), although commercial specifications often refer to alkanoate weight fraction. The analysis by ^{1}H-NMR spectroscopy is greatly facilitated by the excellent solubility characteristics of derivatives with high DSs.

Cellulose alkanoates with a low DS, and with acyl substituents in excess of C-4 (butyrate) are not commercially available, and they require homogeneous phase synthesis conditions. These have become feasible within the last twenty years as a consequence of the discovery of several unconventional cellulose solvent systems. These have recently been the subject of several excellent reviews (Johnson, 1985; Hudson & Cuculo, 1980).

Homogeneously prepared cellulose alkanoates with bulky acyl substituents and with a low DS offer opportunities for lowering the melt temperature of cellulose; for reducing the degree of crystallinity; and for preparing melt-processable derivatives that retain such important material characteristics as water absorption and biodegradability. Because of their often poor solubility characteristics in organic solvents, such derivatives can not easily be analyzed by conventional spectroscopic techniques.

The degree of substitution of cellulose esters with a low DS is determined by solution ^{1}H-NMR spectroscopy using dimethyl sulfoxide (DMSO) as a solvent. This results in a poorly resolved ^{1}H-NMR spectrum

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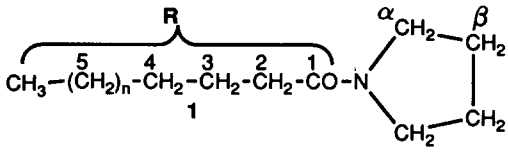
because both higher solution viscosity and extensive hydrogen bonding contribute to significant signal broadening. The large number of hydroxyl protons remain unresolved, and this interferes with quantitative interpretation. Buchanan *et al.* (1991) have recently demonstrated that the hydroxyl protons of cellulose can be shifted into a region of non-interest by the addition of trifluoroacetic acid to the sample solution. This, and operating the spectrometer at an elevated temperature (80°C), does produce improved resolution; however, it is limited by derivative degradation.

Another approach to the analysis of cellulose esters by ^{1}H-NMR spectroscopy involves the re-derivatization of lightly substituted cellulose alkanoates with deuterated acyl substituents (especially acetic acid), which render the derivative soluble in chloroform (Goodlett *et al.*, 1971; Deus & Friebolin, 1991). This method remains indispensable in determining substitution patterns at individual hydroxyl groups of cellulose derivatives, but it is too expensive and cumbersome to be adopted as a routine analytical method.

The alternative to spectral analysis is chemical analysis. This usually involves deacylation followed by quantitative determination of acyl substituents. Mansson and Samuelsson (1981) have recently demonstrated that aminolysis of cellulose esters produces amides of pyrrolidine (structure 1) that can be separated by gas chromatography. This method has, however, not yet been applied to mixed cellulose alkanoates and to those having substituents bulkier than C-2.

It was the objective of the present study to examine the potential for analyzing cellulose alkanoates with large alkanoic acid substituents by aminoloysis/gas chromatography (GC), and to compare the results with those obtained by (solution) ^{1}H-NMR spectroscopy.



Structure 1.

EXPERIMENTAL

Materials

Reagents

Alkanoyl chlorides, pyridine, and pyrrolidine were obtained from Aldrich Chemical Company (Milwaukee, WI, USA), and they were used as received.

Preparation of internal standards: 1-acylpyrrolidines (structure 1)

(a) Acetyl (C-2), propionyl (C-3) and butyryl (C-4) pyrrolidines. The appropriate acid chloride (2 ml) was dissolved in 10 ml of dichloromethane. This was slowly added to an ice-cold solution of pyrrolidine (3 equivalents/acid chloride) in 30 ml of dichloromethane. The rate of the addition of acid chloride was controlled carefully so as to avoid the excessive formation of fumes. The mixture was then allowed to warm to room temperature, and subsequently stirred for 18 h.

After evaporating the solvent under vacuum, diethylether was added to the mixture. The precipitate formed thereby was filtered under suction. The residue was washed several times (3 × 10 ml) with diethylether, and the combined ether filtrates were evaporated. The oily residue was purified by distillation under reduced pressure using 'short path' distillation apparatus.

(b) Hexanoyl (C-6), lauroyl (C-12), Myristoyl (C-14) and stearoyl (C-18) pyrrolidine. The amides were prepared in accordance with the above described procedure except that the work-up procedure was different. They were isolated by washing the reaction mixture with 3 N HCl to remove excess pyrrolidine. The dichloromethane layer was separated and washed with 5% sodium bicarbonate followed by water (till neutral). Myristoyl (C-14) and stearoyl (C-18) pyrrolidine were isolated as solids after solvent evaporation. The other derivatives were isolated as liquids and purified by distillation.

All derivatives raised single peaks by GC and exhibited NMR spectra that are represented in Table 1. Physical property data of the pyrrolidine standards are given in Table 2.

Methods

Selection of internal standard

Internal standards were selected according to the guide given in Fig. 1. Acyl pyrrolidines that separate well under a given set of GC-conditions are collected into groups A, B, and C. An appropriate standard was selected from within a group that contained the acyl pyrrolidine to be determined.

Standard pyrrolidinolysis procedure

A solution of an internal standard (0·5–10 mg/ml) was prepared by dissolving the appropriate pyrrolidine derivative in a 1:1 mixture of pyridine and pyrrolidine. An accurately weighed cellulose alkanoate sample (5–50 mg) was suspended in the internal standard solution (1–2 ml) in a Reactivial (Supelco, Bellefonte, Philadelphia, PA, USA) fitted with a triangular magnetic stir bar. The vial was heated to 80°C in a heating block for 18–72 h, with continuous stirring. After cooling, the reaction mixture was filtered, and a portion of the filtrate was injected into the gas chromatograph. A guide to select the sample weight and the concentration of the internal standard is given in Table 3.

An 18 h reaction time period was found to be

Table 1. ^{1}H-NMR signals of pyrrolidine standards

Standard Acyl pyrrolidine[a]	Chemical shift values[b]						
	CH_3	$C_\alpha H_2$	$C_\beta H_2$	C_2	C_3	C_4	$>C_4$
Acetyl (R = $COCH_3$)	1·97	3·34	1·84				
Propionyl (R = COC_2H_5)	1·06	3·35	1·80	2·20			
Butyryl (R = COC_3H_7)	0·90	3·38	1·84	2·18	1·62		
Hexanoyl (R = COC_5H_{11})[c]	0·86	3·39	1·85	2·20	1·60	1·30	
Lauroyl (R = $COC_{11}H_{23}$)	0·84	3·40	1·87	2·25	1·65		1·23
Myristoyl (R = $COC_{13}H_{27}$)	0·89	3·44	1·90	2·26	1·64		1·25
Stearoyl (R = $COC_{17}H_{35}$)	0·87	3·44	1·90	2·26	1·64		1·25

[a]1-Acyl pyrrolidine with Structure 1.
[b]Numbers are as shown in Structure 1.
[c]^{1}H-NMR spectrum. (see Fig. 3).

Table 2. Physical properties of standards

Standard[a]	Boiling point (°C)	Pressure (mm Hg)	Melting point (°C)	Elemental composition[b]		
				C	H (%)	N
Acetyl (R = –$COCH_3$)	48–48·5	1·5	—	63·5 (63·7	9·36 9·80	11·9 12·4)
Propionyl (R = –COC_2H_5)	58–59	1·5	—	65·8 (66·1	10·6 10·3	11·1 11·0)
Butyryl (R = –COC_3H_7)	61–62	2	—	66·7 (68·0	10·7 10·7	9·81 9·92)
Hexanoyl (R = –COC_5H_{11})	75–77	0·2	—	70·4 (71·0	11·2 11·3	8·21 8·27)
Lauroyl (R = –$COC_{11}H_{23}$)	135–136	0·2	—	74·6 (75·8	12·3 12·3	5·07 5·33)
Myristoyl (R = –$COC_{13}H_{27}$)	—	—	35–38	76·2 (76·8	12·5 12·5	4·29 4·98)
Stearoyl (R = –$COC_{17}H_{35}$)	—	—	50–52	77·6 (78·3	12·9 12·8	3·38 4·15)

[a]1-Acyl pyrrolidine with Structure 1.
[b]Theoretical values are given in parentheses.

adequate for complete aminolysis of esters ranging from acetate (C-2) to butyrate (C-4); a reaction time of 72 h was selected for the higher esters, hexanoate (C-6) to stearate (C-18).

GC analysis procedure

The GC separations were performed in either of two glass (packed) columns using helium as carrier gas. A Varian 3700 gas chromatograph with an HP integrator (model 3394A) was used for analysis. Response factors were determined using the GC chromatogram of the appropriate calibration mixture. Calibration mixtures were prepared by mixing an internal standard with the 1-acylpyrrolidine derivative to be analyzed in approximately equal quantities. The calibration table generated by the integrator was used for subsequent sample analysis. Acetyl (C-2), propionyl (C-3), and butyryl (C-4) derivatives were separated on a Carbowax column in accordance with the method by Mansson and Samuelsson (1981); all other derivatives were separated on an OV-17 column.

DS calculation

The DS was calculated using the following formula:

$$DS = A \times V \times 162/[W(M_w + 70{\cdot}12) - V \times A(M - 1)]$$

Where A = sample concentration in mg/ml;
V = solvent volume used in aminolysis (in ml)
W = mass of the unknown sample (in mg);
M_w = molecular weight of the acyl moiety; and 70·12 is the molecular weight of the pyrrolidine group.

NMR procedure

The solvents used for NMR experiments were deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO). Spectra of chloroform-soluble samples were recorded at ambient temperature. A few drops of trifluoroacetic anhydride were added to each DMSO soluble sample just prior to the recording of their spectra at 80°C.

It is straightforward to calculate the DS from the

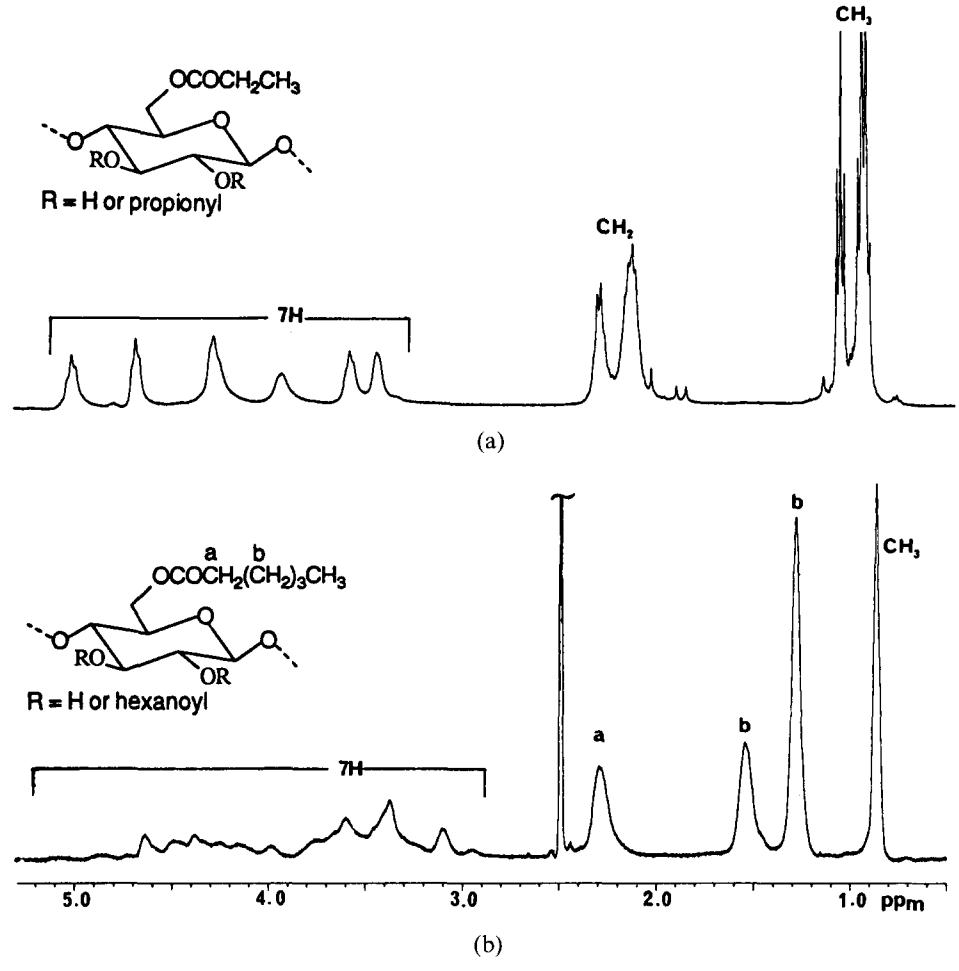


Fig. 1. Typical proton spectra of cellulose alkanoates. (a) Cellulose propionate (DS 2·7) in $CDCl_3$; and (b) cellulose hexanoate (DS 0·6) in d_6-DMSO.

spectra recorded in $CDCl_3$. In DMSO, the cellulose backbone protons (7H) occur in the range of δ 2·5–5·5 ppm. The protons in alkanoyl chains appear in the range of δ 0·5–2·5 ppm. These two regions were integrated separately to obtain DS (see Fig. 1 for peak assignments).

RESULTS AND DISCUSSIONS

Standard procedure

The direct determination of acyl substituents of cellulose alkanoates by aminolysis (with pyrrolidine), followed by the analysis of resulting 1-acylpyrrolidines (structure 1) by GC, required calibration with standards. These acylpyrrolidine standards were prepared from a series of acyl chlorides, ranging in chain length from C-2 to C-18. Physical characteristics of these standards are given in Tables 1 and 2.

The determination of the DS of an unknown cellulose alkanoate involved aminolysis in the presence of an internal standard. GC separations of several mixtures of standard 1-acyl pyrrolidines (Fig. 2) were performed in the method development stage. This allowed the selection of appropriate internal standards which are reported in Table 3. These standards were added to the sample at the beginning of the aminolysis experiment.

The unknown cellulose alkanoate sample may belong to any one of three groups (Table 4). Group A compounds, containing substituents with sizes ranging from C-2 to C-4, were separated on 5% Carbowax using a temperature gradient of 130–140°C. By contrast, alkanoates, with acyl substituents ranging in size from C-4 to C-18 (Table 4), were separated on an OV-17 column using a temperature program range of 130–280°C. Baseline separation within each group, from an internal standard that ranged in peak size from approximately one-third to 3-fold that of the major unknown constituent, produced the best results.

Comparison of ^{1}H-NMR spectroscopy and aminolysis

Proton NMR spectra of cellulose alkanoates produce sharp and well-resolved signals if the sample is soluble in sufficient concentration, and if the viscosity of the

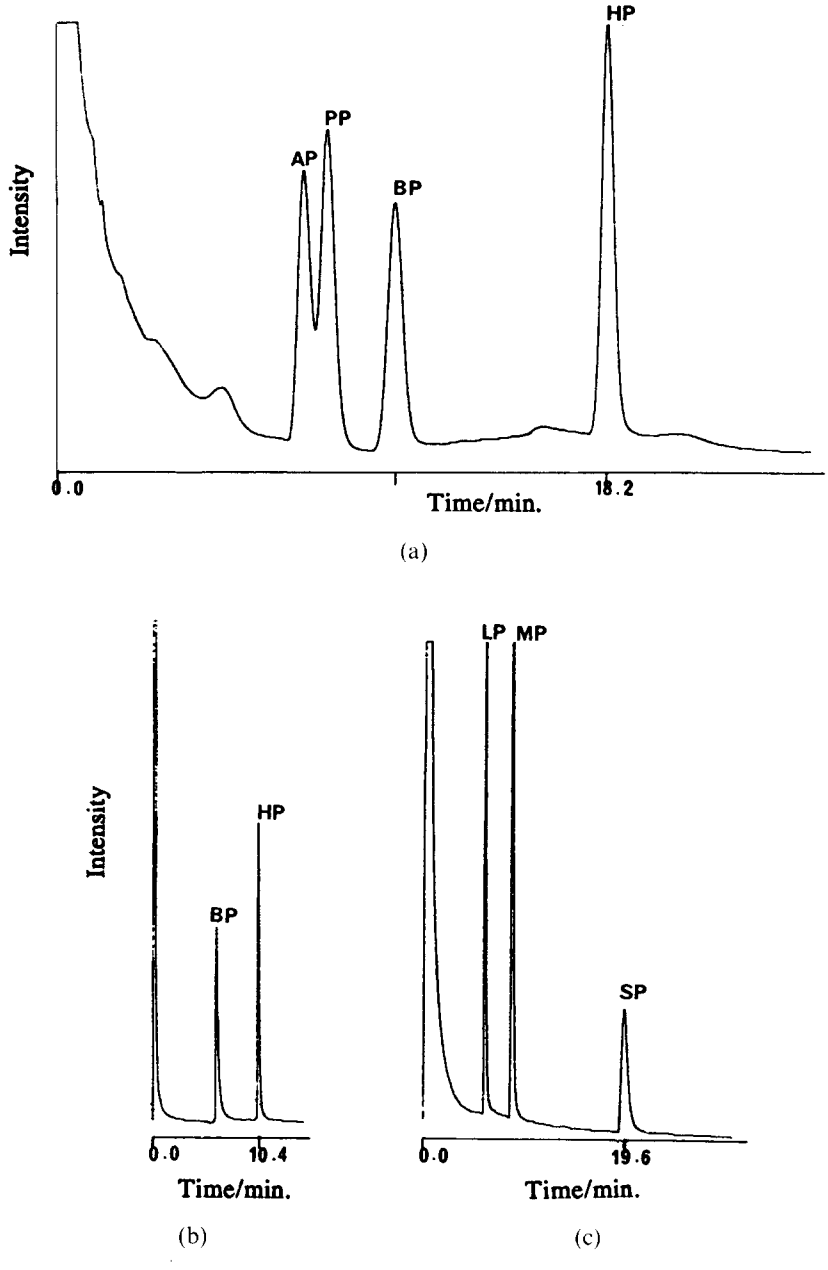


Fig. 2. Gas chromatograms of (a) acetyl (AP), propionyl (PP), butyryl (BP) and hexanoyl (HP) pyrrolidine on 5% cabowax column; (b) butyryl (BP) and hexanoyl (HP) pyrrolidine; and (c) lauroyl (LP), myristoyl (MP) and stearoyl (SP) pyrrolidine on OV-17 column (both b and c).

Table 3. Guide to select sample weights and concentration of internal standard

Size of the ester substituent in cellulose derivative	Estimated DS	Sample (wt/mg)	Concn internal standard	Volume
<C-8	0·1–0·5	50	5	2
	0·5–1·5	25	5	2
	1·5–3·0	10	5	2
>C-8	0·1–0·5	25	0·5	1
	0·5–1·5	10	5	1
	1·5–3·0	5	5	2

solution is low. Small molecules, such as 1-hexanoylpyrrolidine produce well-resolved spectra (Fig. 3). If, however, DMSO or $CHCl_3$-soluble cellulose alkanoates are analyzed, well-resolved spectra are obtained only with those derivatives which have high DSs (Fig. 1(a)). Unresolved spectra are obtained with derivatives with low DSs (Fig. 1(b)).

Alkanoates with DSs lower than 0·4 do not dissolve, even in DMSO. Samples that dissolve partially do not produce reliable spectra. Moreover, the viscosity of DMSO–alkanoate solutions increases progressively with increasing alkanoate size (C-2 to C-18). This produces extremely broad peeks and an unreliable baseline which,

Table 4. Internal standard and GC conditions

Selection guide	Cellulose alkanoate[a]							
	Group A			Group B		Group C		
	Ac	Pro	Bu	Bu	Hex	Lau	Myr	Ste
Internal standard (IS)[a]	Bu	Bu	Pro	Hex	Bu	Myr	Lau	Myr
Retention time of ester (min)	8·25	9·05	11·32	8·83	12·78	6·07	8·67	17·44
GC-column[b]	CW	CW	CW	OV-17	OV-17	OV-17	OV-17	OV-17
Temperature profile								
Initial temp. (°C; min)	130;10	130;10	130;10	130;5	130;3	230;3	230;3	230;3
Temp. gradient (°C/min)	2	2	2	10	10	20	20	20
Final temp. (°C; min)	140;2	140;2	140;2	170;10	170;10	250;6	250;6	250;21
Injector temperature (°C)	200	200	200	250	250	280	280	280
Detector temperature (°C)	250	250	250	250	250	320	320	320
He flow rate (cc/min)	15	15	15	10	10	27	27	27
Sample volume (μl)	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5

[a]Ac, Acetate; Pro, Propionate; Bu, Butyrate; Hex, Hexanoate; Lau, Laurate; Myr, Myristate; Ste, Stearate.
[b]CW, Carbowax; or OV-17 packed column.

in turn, results in erroneous integration and, therefore, the determination of DSs is not reliable. Thus, the qualification of ^{1}H-NMR spectroscopy for analysis of cellulose esters is limited to those derivatives which have acyl substituents with low interference with other signals; and to those having adequate solubility. Aminolysis, by contrast, is independent of solubility or DS. Degrees of substitution as low as 10^{-3} can be detected by this method (eg. Table 5, myristates).

Table 5 provides a comparison of DS data obtained by ^{1}H-NMR spectroscopy and gas chromatography. The results reveal that those derivatives with a low DS (<1·0) are, for the most part, insoluble in solvents useful for ^{1}H-NMR spectroscopy. Whereas derivatives with a high DS produce results by ^{1}H-NMR and aminolysis that lie within ±10% of each other (i.e. cellulose propionate with a DS of 1·6–2·7, Table 5), derivatives with a low DS generate results that may be as much as 50% higher or lower than those obtained by aminolysis/GC. These data are compared graphically in Fig. 4. Whereas a significant agreement is established between the DS data by NMR and GC for DS values of greater than 1·0, significant variability exists for DS levels between 0 and 1·0 (cellulose derivatives with a DS between 0 and 0·3 are virtually insoluble in all common solvents). This is revealed by comparing significance levels for both ranges. Whereas the relation between DS by NMR and DS by GC has an R^2 factor of 0·993

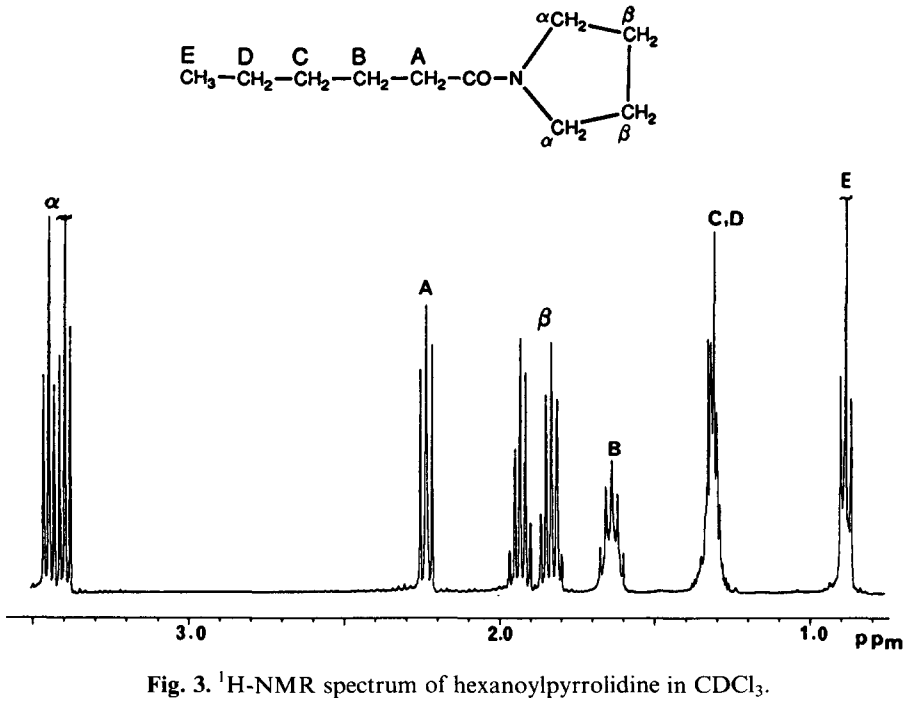


Fig. 3. ^{1}H-NMR spectrum of hexanoylpyrrolidine in $CDCl_3$.

Table 5. Comparison of DS — H-NMR *vs* GC

	DS by				
	^{1}H-NMR			GC	Difference[a] (in % of GC)
	CH_3-signal	α-CH_2-signal	Ave.		
Propionate	IS[b]			0·043	
	IS			0·047	
	IS			0·13	
	0·79	0·74	0·77	0·50	+54
	1·64	1·58	1·61	1·67	−36
	2·40	2·87	2·64	2·60	+15
Butyrate	IS			0·20	
	0·45	0·47	0·40	0·42	−5
	0·51	0·55	0·53	0·60[c]	−12
	—	—	1·77	1·67	
Hexanoate	IS			0·011	
	IS			0·050[d]	
	IS			0·057	
	IS			0·123[d]	
	IS			0·185[c]	
	IS			0·415[c]	
	0·47	0·42	0·45	0·50[c]	−10
	1·0	0·94	1·0	0·70	+43
	0·9	—	9·0	0·87[c]	+3
Laurate	0·3	—	0·3	0·20	+30
	IS			0·55	
	IS			0·93	
	IS			1·06	
	2·00			1·97	
	1·3			1·3	
Myristate	0·8	—	0·8	0·50[c]	+60
	IS			0·36	
	IS			0·002	
	IS			0·001	
Stearate	IS			0·41	

[a]Higher (+) or lower (−) than GC-method.
[b]IS, insoluble in DMSO.
[c]Average of 2 determinations.
[d]Average of 3 determinations.

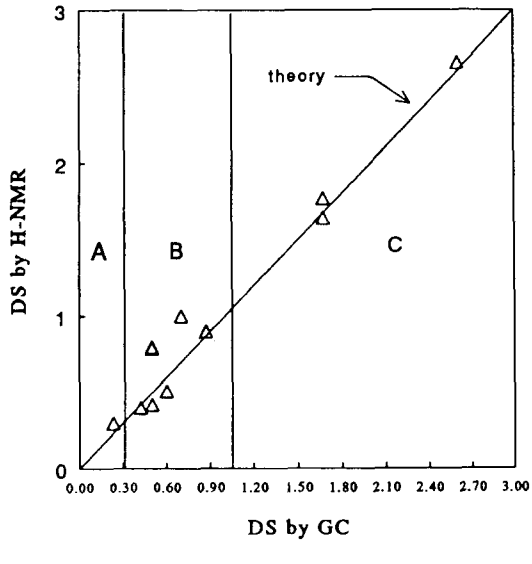


Fig. 4. Comparison of DS data determined by GC and NMR. (A) Insoluble region; (B) low reliability region; and (C) high reliability region. Significance levels, R^2, are 0·760 for region B-data and 0·993 for region C-data.

above a DS of 1·0, this is only 0·760 for the values below a DS of 1·0.

CONCLUSIONS

1. Cellulose alkanoate analysis can be performed by either proton NMR spectroscopy or by aminolysis (with pyrrolidine), followed by gas chromatographic separation of resulting 1-acyl pyrrolidines. Whereas conventional esters, with high DSs and acyl substituents exhibiting few signals in the ^{1}H-NMR spectrum, can be analyzed with reliability by NMR spectroscopy, unconventional esters, prepared by homogeneous phase reaction of cellulose with bulky alkanoyl groups to low DSs, require chemical analysis for reliable determination of DS.
2. Aminolysis/GC is a rapid method suitable for semi-microanalysis over a wide range of DSs and substituent types (i.e. C-2 to C-18).

3. The correlation of DS values by NMR as opposed to GC is significant above a DS level of 1·0; and it is significantly variable at DS levels below 1·0.

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