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Note

Preparation of poly(acyl)chitosans

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During studies on applications of chitin for industrial purposes[1], the need arose for derivatives of chitosan that are soluble in organic solvents Danilov and Plisko[2] obtained an ethyl ether of chitin by treatment with ethyl chloride in an autoclave for 10 h Fully methylated chitin was prepared[3] from chitosan by repetition of acylation and methylation procedures Only a few attempts have been made to solubilize chitosan in organic solvents by chemical modification[4]

Acylation, especially with long-chain fatty acids, is a valuable method for changing the solubility properties of sugars Much literature exists covering various aspects of acylation of hexitols[5] and of sucrose[6] to yield potentially surface-active derivatives of sugars Recently, Hirano and co-workers reported[7] the preparation

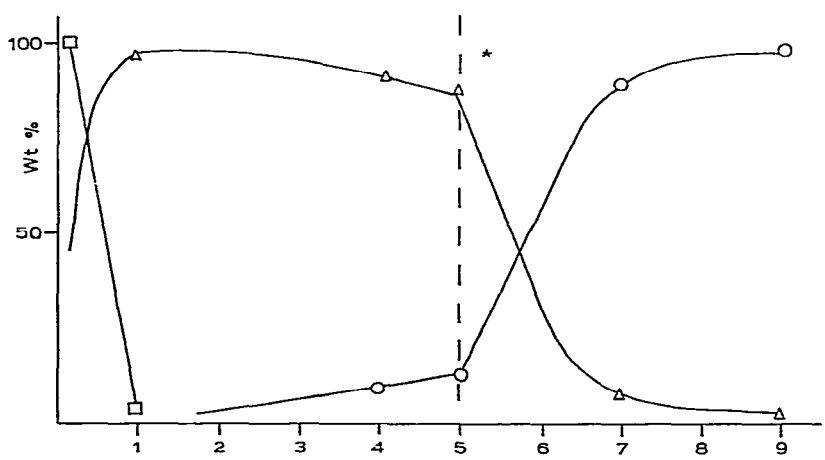

Fig 1 Treatment of chitosan with dodecanoyl chloride Chitosan (1 6 g) was boiled under reflux with dodecanoyl chloride (15 4 g) in a mixture of pyridine (46 mL) and chloroform (34 mL) Additional dodecanoyl chloride (7 7 g) in chloroform (6 4 g) was added (*) after 5 h Aliquot samples were poured into methanol, the resultant precipitates were washed with methanol, and extracted with chloroform (chloroform-soluble fraction, —○—) and then extracted with 0 1% hydrochloric acid (chitosan fraction, —□—) The residue (chloroform-insoluble fraction, —△—) and the extracts were weighed and analyzed

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TABLE I

ANALYTICAL DATA FOR POLY(ACYL)CHITOSANS

Acyl group	*C*	*H*	*N*	*Dec* [a]
Hexanoyl (found)	65 11	9 19	2 54	260–327°
Calc for $C_{30}H_{51}NO_8$[b]	65 05	9 30	2 53	
Dodecanoyl (found) (chloroform-soluble fraction)	72 68	11 66	1 84	256–300°
Calc for $C_{54}H_{99}NO_8$[b]	72 83	11 23	1 57	
Dodecanoyl (found) (chloroform-insoluble fraction)	71 41	11 30	2 00	
Calc for $C_{43}H_{77}NO_7$[c]	71 71	10 80	1 95	
Tetradecanoyl (found)	74 61	11 77	1 38	253–294°
Calc for $C_{62}H_{115}NO_8$[b]	74 33	11 49	1 40	

[a]Temperature range for thermal decomposition [b]Four mol of acyl group per monosaccharide residue [c]Three mol of acyl group per monosaccharide residue

of partially acylated chitosan derivatives by treating aqueous acetic acid–methanol solutions of chitosan with acid anhydrides at room temperature, but, as with chitin, the products were soluble neither in organic solvents nor in water We now report a convenient method for preparation of fully acylated chitosan derivatives that are soluble in organic solvents

Chitosan was boiled under reflux with a large excess of dodecanoyl chloride in a mixture of dry pyridine and dry chloroform An additional amount of dodecanoyl chloride was added after 5 h Aliquots were taken every h from the mixture and separated into 3 fractions chloroform-insoluble, chloroform-soluble, and the chitosan fraction The progress of the reaction was thus monitored (Fig 1)

After 1 h, most of the product was chloroform-insoluble and it contained only 1 0% of free chitosan The presence of NH, *N*-acyl, and *O*-acyl groups in the chloroform-insoluble fraction was shown by i r spectral absorptions (KBr) at 1540 (NH, lit.[8] 1540 cm^{-1}), 1670 (C=O of *N*-acyl, lit [9,10] 1650), and 1745 cm^{-1} (C=O of *O*-acyl, lit [10] 1750 cm^{-1}) Analytical data (Table I) suggested that the degree of substitution was ~3 per monosaccharide residue

After 9 h, the mixture had become completely homogeneous; the precipitate obtained by addition of methanol was soluble in chloroform, benzene, ether, and pyridine Analytical data (Table I), and the ratio of methyl and methylene protons of the dodecanoyl group (δ 3 0–7 0) to the methine and methylene protons of the sugar residues (δ 0 7–7 0), as calculated by integration of protons in the n m r spectrum, suggested that the d s by dodecanoyl groups was ~4 per monosaccharide residue

No absorption was present in spectral regions associated with NH (1540 cm^{-1}),

OH (3300–3500), and C=O of NHCOR (1670), but a new absorption at 1710 cm^{-1} [C=O of $N(COR)_2$, lit.[12] 1694 and[11] 1683 cm^{-1}] was present These results thus suggested that the chloroform-soluble fraction had two *O*-dodecanoyl groups and one *N*-dodecanoyl(dodecanamido) group per monosaccharide residue

Sircar and co-workers described[13] the formation of an α-propionylpropionyl group as a side reaction accompanying the main reaction of cellulose with propionyl chloride and pyridine Gas-chromatographic examination of the chloroform-soluble, acylated chitosan derivatives showed the presence of only one peak corresponding to dodecanoic acid, and no additional peak[13] ($R\text{-}CO\text{-}CH_2R$) derived from an α-acylacyl group [CO-CH(COR)-R′] was present The i r spectrum of this chloroform-soluble fraction showed no absorption[13] at 1800 cm^{-1} for the ketone group of an α-acylacyl substituent These facts support the foregoing conclusion

As further supporting evidence, 2-deoxy-2-dodecanamido-1,3,4,6-tetra-*O*-dodecanoyl-β-D-glucopyranose (**1**) was converted into 2-deoxy-1,3,4,6-tetra-*O*-dodecanoyl-2-*N*-dodecanoyl(dodecanamido)-β-D-glucopyranose (**2**) by further acylation with an excess of dodecanoyl chloride in the presence of pyridine and chloroform[14]. No signal for the NH proton (doublet at δ 6 27) of **1** was present in the n m r spectrum of **2**, and no absorption in the spectral region associated with the NH group of an NHCOR substituent (1512 and 3240 cm^{-1})[14] was observed in the i r spectrum of **2** These facts suggest that, under these reaction conditions, the additional acyl group ($R\text{-}CO^+$) did not attack the α-position of the acyl group in the NHCOR and/or O-COR substituent, but instead attacked the nitrogen atom of the NH-COR substituent to give the $N(COR)_2$ group Detailed analysis of the n m r and i r spectra of **1** and **2** eliminate the possibility that an α-dodecanoyldodecanoyl group had been formed under these conditions

The chloroform-soluble fraction was eluted by chloroform from a column of Sephadex LH-20 just at the void volume It is thus clear that the fraction did not contain free dodecanoic acid as an inclusion compound In a separate experiment with standard dodecanoic acid on an identical column of Sephadex LH-20, the acid was eluted far beyond the void volume

Under the same conditions of reaction, chitosan reacted with tetradecanoyl chloride or hexanoyl chloride to afford chloroform-soluble chitosan derivatives (Table I) Evaporation of the solvent from solutions of these poly(acyl)chitosans in chloroform left a rubberlike, thin film

EXPERIMENTAL

General methods — The temperature range of thermal decomposition was determined with a Differential Thermal Analyser $TRDA_3$-L (Cho Balance Corp) N m r spectra were recorded with a Hitachi R-22 spectrometer (in $CDCl_3$ with internal Me_4Si), and i r spectra with a Shimadzu IR-27 spectrometer (KBr pellets) Gel-filtration column chromatography was performed with a glass column (9 × 120 mm) packed with Sephadex LH-20 Saponification was performed with 2 5M

potassium hydroxide–ethanol, and boiling under reflux was continued for 2 5 h Fatty acids were extracted with ether from the acidified solutions and the ether extracts were esterified with methanol and boron trifluoride etherate Gas chromatography was performed with a Shimadzu GC-3AF chromatograph equipped with a hydrogen flame-ionization detector, and a stainless-steel column (3 mm × 3 m) packed with 15% DEGS on Neopak 1A The operating temperature was 205° and nitrogen was the carrier gas

Poly(dodecanoyl) chitosan — Chitosan was prepared according to the method of Horton and Lineback[15] from crab shells Reprecipitated chitosan (1 69 g) was soaked in pyridine (30 mL) for 3 days and then the pyridine was evaporated off under diminished pressure below 40° The chitosan was homogenized with 46 mL of pyridine, and chloroform (21 mL) was added To this stirred, cold mixture (−10°), dodecanoyl chloride[16] (15 4 g) in chloroform (12 7 mL) was added dropwise The mixture was boiled for 5 h under reflux, cooled again, and dodecanoyl chloride (7 7 g) in chloroform (6 4 mL) added After boiling for a further 4 h, the mixture was poured into methanol (300 mL) Repeated precipitations were conducted with 50 mL of ether and 600 mL of acetone, yield 5 1 g (57 5%)

Anal Calc for $C_{54}H_{99}NO_8$ C, 72 83, H, 11 23, N, 1 57 Found C, 73 13, H, 11 82, N, 1 84

Poly(hexanoyl) chitosan. — A procedure similar to that described for the preparation of poly(dodecanoyl)chitosan was used, starting with chitosan (320 mg) and hexanoyl chloride (1 90 g), and then an additional 1 95 g of the chloride Boiling under reflux was continued for a total of 6 h for acylation Reprecipitation of the crude product from ether and methanol furnished 602 1 mg (54 7%) of poly(hexanoyl)chitosan, for analytical data, see Table I

Poly(tetradecanoyl)chitosan — A similar procedure, starting with chitosan (161 mg), tetradecanoyl chloride (1 63 g), and an additional 1 60 g of the chloride, yielded 886 mg (62 8%) of poly(tetradecanoyl)chitosan Reprecipitation was performed with chloroform and methanol, for analytical data, see Table I

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