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The *N*-Permethylation of Chitosan and the Preparation of *N*-Trimethyl Chitosan Iodide

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SUMMARY

Chitosan was N*-permethylated by reaction with formaldehyde and sodium borohydride under controlled conditions (pH 4·0, 15°C, reaction times 12 and 8 h, respectively). The* N*-permethylated chitosan was reacted with methyl iodide at 35°C and* N*-trimethyl chitosan iodide with a quaternary nitrogen degree of 60% was obtained. This material may have uses as an antibiotic and an ion exchange material.*

INTRODUCTION

The conditions for the preparation of *N*-methylene chitosan from formaldehyde and crab chitosan have been recently reported by Hirano *et al.* (1979), who also showed that the product was a suitable medium for gel chromatography. The rate of the reaction between chitosan and formaldehyde has been found to depend upon formaldehyde concentration, temperature and pH (Synowiecki *et al.*, 1982). Other authors have found that *N*-methylene chitosan is suitable for immobilizing enzymes.

Muzzarelli *et al.* (1983) have reported the preparation of *N*-alkyl chitosans. In those compounds the extent of intermolecular hydrogen bonding is reduced by the presence of substituents and therefore they swell in water better than chitosan. Films were easily cast from acetic acid solutions.

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So far, the preparation of *N*-permethylated chitosan has not been reported, although both *N*-trimethyl chitosan iodide and *N*-trimethylglycol chitosan have been described (Muzzarelli, 1977; Kokufuta *et al.*, 1982). According to Nud'ga *et al.* (1973), the addition of methyl iodide to a reaction medium comprising triethylamine and chitosan (molar ratio of methyl iodide to chitosan, 6:1) gives a methylated chitosan with a 64% composition of quaternary nitrogen.

N-Trimethyl chitosan iodide was reported to have a significant antibacterial effect on Gram-positive bacteria but only a very weak effect on Gram-negative bacteria. It gives a precipitate with horse serum but does not influence haemolysis and coagulation of horse erythrocytes (Hatta *et al.*, 1950). *N*-Trimethylglycol chitosan iodide has been studied with respect to its ability to form polysalt complexes with anionic exchangers and to immobilize *Nitrosomonas europaea* cells with the aid of poly(vinyl alcohol)sulphate (Kokufuta *et al.*, 1982*a*, *b*). In view of the increasing interest in quaternary alkyl derivatives of biopolymers (Grollier *et al.*, 1982) and synthetic polymers (Dudley & Williams, 1981), we have carried out the preparation of *N*-trimethyl chitosan iodide by a simple method.

EXPERIMENTAL

Chitosan and reagents

Chitosan from *Euphausia superba* was supplied by the Fisheries Central Board, Szczecin, Poland. The sample had a degree of deacetylation of 58 ± 4%, a pK value of 6·6 and gave X-ray diffraction peaks at 8° 58′ and 19° 58′ (2θ). Other reagents were supplied by Hoechst, Darmstadt, Federal Republic of Germany.

Alkalimetry

Titrations were carried out under nitrogen with 0·1 M NaOH, on solutions of polymers (0·5 g) in 0·3 M HCl (20 ml).

Infrared spectrometry

Chitosan powders were ground with i.r.-grade KBr in an agate mortar. Spectra were recorded with the Perkin-Elmer Model 299-B spectrometer,

with translucent discs obtained by pressing the ground material with the aid of the Perkin-Elmer press.

X-ray diffraction spectrometry

Chitosan powders were milled and sieved to pass a 200-mesh net and compacted under pressure. The samples were exposed to the primary beam of the Ni-filtered CuK_α radiation from a Jeol X-ray diffractometer.

Atomic absorption spectrometry

Analyses were carried out with the Perkin-Elmer 2380 spectrometer equipped with flame and HGA-400 graphite atomizers. Metal ion solutions of ion concentrations 0·25, 0·50, 1·00 and 2·00 mM were prepared; 50 ml of these solutions were shaken with 200 mg of the polymer powder for 24 h on a laboratory shaker operating at 80 rpm. Measurements of ion concentrations were made after 1 h and 24 h shaking.

Circular dichroism spectropolarimetry

A Jasco spectropolarimeter model J-500-A was used, with wavelength expansion of 10 nm cm^{-1}, chart speed 1 cm min^{-1}, sensitivity 5 m° cm^{-1}, time constant 4 s and a cell pathlength of 1 cm. The polymer concentration was 0·05% in 0·1% acetic acid. With the exception of $HgCl_2$ and $Pb(NO_3)_2$, metals were added in the sulphate form. Ion concentrations were 0·2, 0·6, 1·0 and 2·0 mM.

Nuclear Magnetic Resonance spectrometry

The ^{13}C spectra were obtained at 35°C with a 20 MHz Varian CFT-20 spectrometer equipped with a 10 mm probe. The solutions were prepared in D_2O (90–100 mg in 2 ml, 99·7% D), and their pH values were adjusted to less than 5 by concentrated DCl. The experimental conditions were: 90° pulse, 2K data points, Fourier number 8K, 100–300K pulses; the chemical shifts were measured against a CH_3OH internal standard 50·04 ppm from the internal TMS.

RESULTS AND DISCUSSION

Preparation of *N*-methyl chitosans

The chitosan powder (20 g, corresponding to 65 m mol of NH_2) was suspended in water (2 litres) and glacial acetic acid (20 ml) was added while stirring. After the chitosan had dissolved, an aqueous solution of formaldehyde was added. The level of formaldehyde was twice the stoichiometric requirement for the reaction. After 30 min, the pH was increased to 4·5 by the addition of NaOH. Hydrogenation was performed by the stepwise addition of $NaBH_4$ (5 g) dissolved in water (50 ml) over a period of 1 h, while stirring continuously. After a further hour, the pH had increased spontaneously to 5·5, and it was later adjusted to 10 to insolubilize the *N*-methyl chitosan. The product was washed with water to neutrality and then extracted with ethanol and diethyl ether in a Soxhlet apparatus to remove excess aldehyde and inorganic products.

N-Methyl chitosan dissolves in dilute acetic acid and retains the characteristic film forming property of chitosan; it is a basic substance like chitosan, with a pK value of 6·7. The percentage of acetamido groups obtained from infrared and alkalimetry was 36%, while the percentage of secondary amine groups determined by circular dichroism and alkalimetry was 29%, so that the percentage of primary amine groups (obtained by difference) was 35%. X-ray diffraction peaks for *N*-methyl chitosan were found at angles: $2\theta = 8° 36'$ and $20° 30'$ compared with $8° 58'$ and $19° 58'$ for the original chitosan.

Preparation of *N*-dimethyl chitosan (*N*-permethylation of chitosan)

This preparation differs substantially from the preparation of *N*-methyl chitosan in terms of aldehyde concentration and reaction times. Chitosan from *Euphausia superba* (50 g, 100–200 mesh) was suspended in 2 litres of water and dissolved by adding 50 ml of glacial acetic acid. The resulting pH was 3·2. A 35% aqueous solution of formaldehyde (500 ml) was then added with vigorous stirring and a *N*-methylene chitosan gel was immediately obtained. Gel formation of *N*-methylene chitosan from chitosan acetate was found to depend on the amount of formaldehyde, the molecular size of the polymer and the amount of aldehyde. When the chitosan from Dungeness crab was used instead of the chitosan from *Euphausia superba*, gel formation took at least 24 h and the gels

were not so rigid as those obtained with the chitosan from *Euphausia superba*, because of the lower average molecular size (170 000 daltons for the chitosan from Dungeness crab, against 2 000 000 daltons for chitosan from *Euphausia superba*).

After standing for 12 h, sodium borohydride (13 g) was added to the mixture over an 8-h period, the temperature of the reaction medium being kept below 20°C (preferably about 15°C) and the pH value around 4·0. After completing the reduction, NaOH was added to raise the pH to 9·0; the gel was thoroughly washed with water and then collapsed by the addition of acetone. This preparation can be carried out with sulphuric acid (17 ml, conc.) instead of acetic acid, in which case the product is a white powder instead of a gel. The final product was extracted with diethyl ether in a Sohxlet apparatus and was found to be insoluble in acetic and hydrochloric acids.

Figure 1 shows the ^{13}C-n.m.r. spectrum (20 MHz, D_2O) of dimethyl chitosan. Assignments were made by comparison with spectra of D-glucosamine, chitosan, 2-acetamido-2-deoxy-D-glucose and chitin (Tsuka & Inoue, 1981; Gagnaire *et al.*, 1982; Saito *et al.*, 1982). The methyl signal of the *N*-Me group is at about 42 ppm. C-1 of *N*-methylated residues shifts 2·5 ppm with respect to unmodified residues. From ratios of the areas of the pertinent signals in the C-1 region, the degree of *N*-methylation was about 40%, and the degree of *N*-acetylation about 29%.

Preparation of *N*-trimethyl chitosan iodide

N-Dimethyl chitosan (5 g) was kept at 80°C overnight and then was added to a mixture of acetonitrile (100 ml), and methyl iodide (2·5 ml) under anhydrous conditions and kept at 35°C for 30 h while stirring continuously. The product was extracted with diethyl ether in a Sohxlet apparatus. The yield was 6·1 g. This product (1 g) was added to a mixture of water (50 ml), HCl (60 ml, conc.) and CCl_4 (5 ml), and was titrated with 0·025 M potassium iodate using standard procedures. The end point was found at 25·5 ml, and indicated an iodine content of 16%. On the basis of the elemental analysis and of the degree of deacetylation of the original chitosan, the amino groups were substituted as follows: 40% acetamido; 35% trimethylammonium iodide and 25% monomethylamino and dimethylamino. The degree of quaternary ammonium substitution calculated on the primary amino groups was

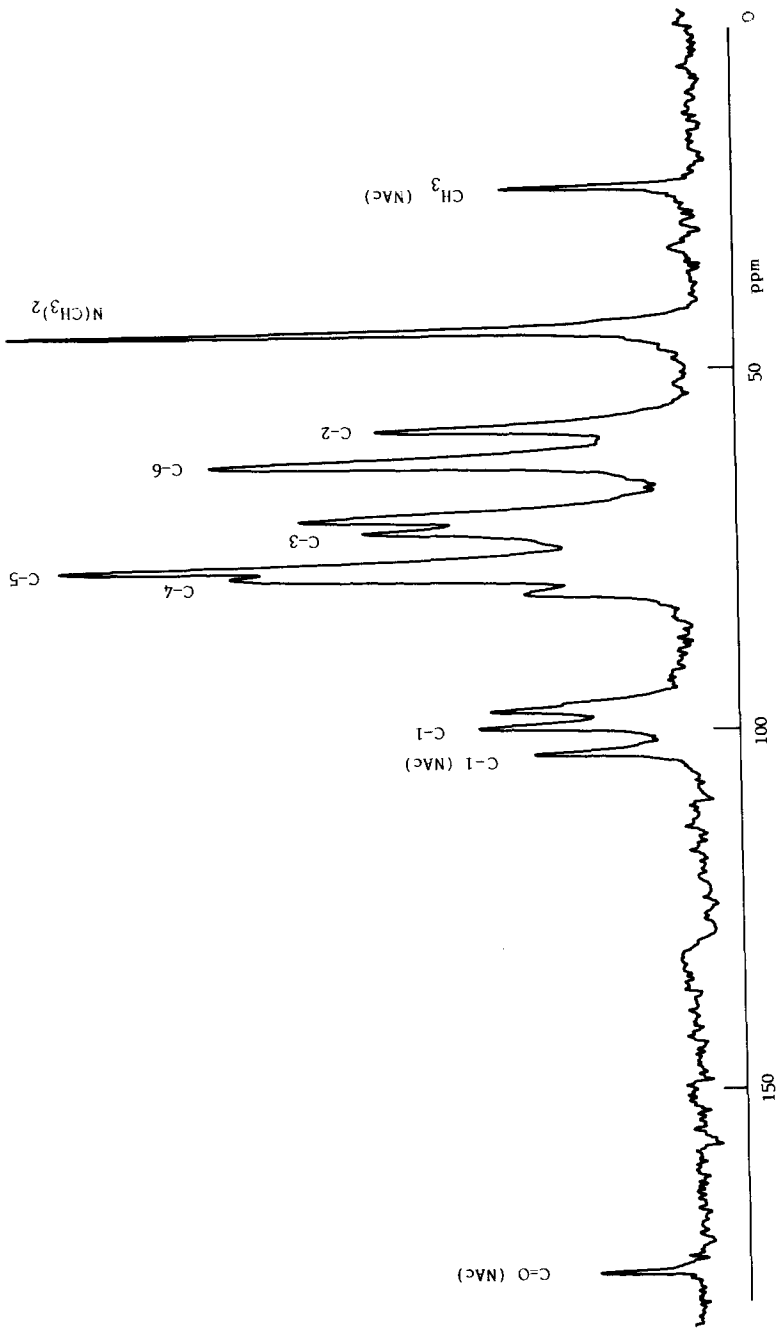

Fig. 1. ^{13}C-n.m.r. spectrum (20 MHz, D_2O) of dimethyl chitosan.

about 60%. *N*-Trimethyl chitosan iodide is insoluble in water but it swells, as expected for a undegraded polymer.

Reaction scheme

The reaction scheme is the following:

$$\text{Chit—NH}_2 + \text{CH}_2\text{OH}^+ \rightleftharpoons \underset{\displaystyle \text{OH}}{\overset{\displaystyle +}{\text{CH}_2\text{NH}_2}\text{—Chit}} \underset{+\,\text{H}_2\text{O}}{\overset{-\,\text{H}_2\text{O}}{\rightleftharpoons}} \text{CH}_2{=}\overset{+}{\text{N}}\text{H—Chit}$$

$$\rightleftharpoons \overset{+}{\text{CH}_2}\text{—NH—Chit} \xrightarrow{\text{BH}_4^-} \underset{N\text{-methyl chitosan}}{\text{CH}_3\text{—NH—Chit}} \xrightarrow{\overset{+}{\text{C}}\text{H}_2\text{OH}} \underset{\displaystyle \text{OH}}{\text{CH}_2\text{—}\overset{+}{\text{N}}\text{HCH}_3\text{—Chit}}$$

$$\underset{+\,\text{H}_2\text{O}}{\overset{-\,\text{H}_2\text{O}}{\rightleftharpoons}} \text{CH}_2{=}\overset{+}{\text{N}}\text{CH}_3\text{—Chit} = \overset{+}{\text{C}}\text{H}_2\text{—NCH}_3\text{—Chit} \xrightarrow{\text{BH}_4^-} \underset{N\text{-dimethyl chitosan}}{(\text{CH}_3)_2\text{N—Chit}}$$

$$(\text{CH}_3)_2\text{N—Chit} + \text{CH}_3\text{I} = \underset{N\text{-trimethyl chitosan iodide}}{(\text{CH}_3)_3\overset{+}{\text{N}}\text{—Chit I}^-}$$

Transition element chelation by *N*-methyl chitosan and *N*-dimethyl chitosan

Both *N*-methyl chitosan and *N*-dimethyl chitosan are chelating polymers. Evidence of the chelating ability of *N*-methyl chitosan was obtained by circular dichroism spectropolarimetry on 0·05% solutions in 0·1% acetic acid, and is presented in Table 1. The typical Cotton band of chitosan at 210 nm is altered and shifted to higher values when *N*-methyl chitosan interacts with Cr^{3+}, Cu^{2+}, Hg^{2+} and Pb^{2+}, the effects being quite dramatic in the case of 0·2 mM Cu^{2+} at pH 6·0 for which new bands at 240 and 275 appear (Table 1). Mercuric ions also strongly interact with *N*-methyl chitosan showing a positive band at 260 nm and the simultaneous disappearance of the negative band at the higher (2 mM) concentration tested. Table 2 shows the capacity and collection percentages of *N*-permethylated chitosan for various ions. With experimental error, the same values are obtained with *N*-methyl chitosan and *N*-dimethyl chitosans prepared either in sulphuric acid or acetic acid. Linear isotherms for Cr^{3+} and for Cu^{2+} and a slight increase in capacity with time

TABLE 1

Circular Dichroism Data on *N*-dimethyl Chitosan Solutions (0·05% in 0·1% acetic acid) in the Presence of Metal Ions at Various Concentrations

Band type	*Ion concentration (mM)*																					
	Cr^{3+}, pH 5·5				*Co^{2+}, pH 5·1*				*Cu^{2+}, pH 4·5*			*Cu^{2+}, pH 6·0*			*Hg^{2+}, pH 5·5*				*Pb^{2+}, pH 5·5*			
	0·2	*0·6*	*1·0*	*2·0*	*0·2*	*0·6*	*1·0*	*2·0*	*0.2*	*0·6*	*1·0*	*0·2*	*0·6*	*1·0*	*0·2*	*0·6*	*1·0*	*2·0*	*0·2*	*0·6*	*1·0*	*2·0*
+, λ	no	no	no	no	no	no	no	no	no	no	no	240	240	245	no	no	no	260	no	no	no	no
+, θ	no	no	no	no	no	no	no	no	no	no	no	1·1	2·2	9·9	no	no	no	2·5	no	no	no	no
−, λ	210	212	214	220	210	210	210	210	210	212	215	275	280	290	212	215	219	no	221	no	no	no
												210	212	213								
−, θ	22·7	21·3	17·3	9·7	24·2	24·2	25·2	26·7	23·4	22·0	16·2	5·8	10·5	18·0	22·7	17·5	12·3	no	9·4	no	no	no
												23·1	21·8	22·5								

Key: λ, wavelength at maximum band height (nm); θ, 10^{-4} degrees $cm^2\,dmol^{-1}$ (at maximum band height); no, band present; +, positive Cotton band; −, negative Cotton band.

TABLE 2
Ion Collection Percentages and Capacities for *N*-permethylated Chitosan (200 mg in 50 ml, after 1 h and 24 h contact, at 25°C)

Ion (*mmol litre*$^{-1}$)	*After 1 h*				*After 24 h*		
	pH	*Uptake* (%)	*Capacity*		*Uptake* (%)	*Capacity*	
			mg g^{-1}	*mmol g*$^{-1}$		*mg g*$^{-1}$	*mmol g*$^{-1}$
Chromium III							
0·25	6·2	90	2·92	0·056	98	3·18	0·06
0·50	6·4	89	5·78	0·111	93	6·04	0·116
1·00	6·5	96	12·47	0·24	93	12·08	0·23
2·00	6·4	95	24·69	0·475	96	24·95	0·48
Manganese II							
0·25	6·2	0	0	0	22	0·75	0·013
0·50	6·2	0	0	0	20	1·37	0·025
1·00	6·4	0	0	0	4	0·55	0·010
2·00	6·7	0	0	0	6	1·65	0·030
Cobalt II							
0·25	6·8	13	0·48	0·008	31	1·14	0·019
0·50	6·7	9	0·66	0·011	16	1·18	0·020
1·00	6·7	5	0·74	0·012	16	2·36	0·040
2·00	6·7	14	4·12	0·070	11	3·24	0·055
Nickel II							
0·25	7·00	0	0	0	0	0	0
0·50	7·00	20	1·46	0·025	10	0·36	0·006
1·00	7·00	50	7·33	0·125	50	7·33	0·125
2·00	6·8	75	22·01	0·375	70	20·54	0·350
Copper II							
0·25	6·7	100	3·97	0·062	100	3·97	0·062
0·50	6·6	100	7·94	0·125	100	7·94	0·125
1·00	6·1	82	13·02	0·205	100	15·88	0·250
2·00	5·5	92	29·25	0·46	94	29·86	0·470
Uranyl II							
0·25	6·6	96	14·28	0·06	97	14·43	0·06
0·50	6·0	84	24·99	0·105	89	26·48	0·111
1·00	5·6	76	45·22	0·19	91	54·15	0·22
2·00	5·5	74	88·07	0·37	90	107·11	0·45

are found. In comparison to chitosan itself, the values for the alkyl chitosans are lower for Mn^{2+} and Co^{2+}, but are higher for Cr^{3+} and UO_2^{2+}.

CONCLUSIONS

N-Trimethyl chitosan iodide has been prepared under milder conditions than those reported by previous authors. This product may have useful antibiotic, ion exchange and flocculating properties and has applications in the cosmetic industry. Moreover, the quaternary ammonium derivatives of amino sugars and proteins are necessary for their determination by mass spectrometry. *N*-Trimethyl chitosan salts seem suitable for further studies on the anticholesterolemic action of chitosan, because they have similarities to cholestyramine, a well-known anticholesterolemic agent. The enhanced selectivity toward transition metal ions supports the view that appropriate derivatization of chitosan leads to more selective chelating polysaccharides.

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