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Chlorination of chitin with sulfuryl chloride under homogeneous conditions

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Chitin was chlorinated with sulfuryl chloride under homogeneous conditions in LiCl–*N*,*N*-dimethylacetamide. Chlorodeoxychitins were obtained in the reactions at temperatures from −30 to 70°C. The regioselective replacement of C-6 hydroxyl groups with chlorine atoms occurred in the reaction for 4 h at −20°C while C-3 hydroxyl groups were also replaced above 0°C when the molar ratio of sulfuryl chloride to the repeating unit of chitin was 15. The yield of chlorodeoxychitin decreased from 90 to 60% with the rise in reaction temperature from −30 to 60°C and dropped to 27% at 70°C. All the chlorodeoxychitin samples were soluble in formic acid. © 1997 Elsevier Science Ltd

INTRODUCTION

We reported previously the chlorination (Sakamoto *et al.*, 1994) and bromination (Tseng *et al.*, 1995a, b) of chitin under homogeneous conditions in solvent systems consisting of lithium chloride or bromide and *N*,*N*-dimethylacetamide (DMA). For halogenation reagents, *N*-chlorosuccinimide (NCS), *N*-bromosuccinimide and tribromoimidazole (Br_3Im) were used in combination with triphenylphosphine (PPh_3). In these halogenations of chitin, the degree of substitution by halogen was close to 1 at the highest and C-6 hydroxyl groups were replaced regioselectively with halogen atoms. These results are different from those of the halogenations of cellulose with NCS–PPh_3 (Furuhata *et al.*, 1992) and Br_3Im–PPh_3 (Furuhata *et al.*, 1995) under homogeneous conditions where C-3 hydroxyl groups were also replaced with halogen atoms at a rate slower than that for C-6 hydroxyl groups.

The reason for the difficulty in the substitution of C-3 hydroxyl groups of chitin can be ascribed to the steric hindrance of bulky C-2 acetamido groups. In the halogenation with the above-mentioned reagent systems, the important step is considered to be the formation of triphenylphosphonium ester (Classon *et al.*, 1981; Hodosi *et al.*, 1992) which is susceptible to nucleophilic attack by a halide ion to give a halodeoxy compound and triphenylphosphine oxide. The formation of the bulky phosphonium ester at C-3 will be sterically hindered by the adjacent acetamido group. An intermediate moiety of smaller size, on the other hand, may be formed at C-3 and a 3-deoxy-3-halo unit will result in that case through the nucleophilic attack by a halide ion. Sulfuryl chloride is one of the halogenation reagents that satisfy such a requirement.

In this paper, we describe the chlorination of chitin with sulfuryl chloride under homogeneous conditions in LiCl–DMA. Effects of reaction conditions on the extent of chlorination were studied. Under appropriate conditions, C-3 hydroxyl groups were replaced with chlorine atoms along with C-6 hydroxyl groups.

EXPERIMENTAL

Materials

A chitin sample (generous gift of Katokichi Co.) was purified according to the method described by Tokura (1983). The degree of deacetylation (DeAc) of purified chitin sample was 9.7%, which was determined from the atomic ratio of carbon to nitrogen. The calculated average molar mass of repeating units (PRUs) was 199.12. Sulfuryl chloride was purified by distillation just before use (b.p. 69°C). DMA was dried with calcium hydride and distilled under reduced pressure. Lithium chloride was dried under reduced pressure in a Schlenk tube.

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Chlorination with sulfuryl chloride

For the chlorination, chitin (0.5 g) was dissolved in LiCl–DMA (5 g/60 ml) according to the procedure described in the previous paper (Sakamoto *et al.*, 1994) and sulfuryl chloride was added with a dropping funnel to the solution at −40°C. The molar ratio of sulfuryl chloride to PRU was in the range 5–30 (mostly about 15). The final volume of the reaction solution was adjusted to 100 ml with DMA. The solution was stirred vigorously for 15 min at −40°C and then kept for a prescribed period (1–8 h) at a predetermined temperature (from −30 to 70°C). The color of the solution was yellow at −40°C and became orange to red-brown at higher temperatures. In experiments studying the effect of reaction time, aliquots were pipetted from the reaction solution at predetermined time intervals. After the reaction, the solution was poured slowly into ten times volume of acetone. The separated material was washed with acetone and methanol until the washings were not colored. After treatment with a sodium carbonate solution (pH 11.4) for 12 h, the sample was dialyzed against running water for 3 days, against distilled water for 2 days and lyophilized.

Analyses

The DeAc values of chlorodeoxychitin samples were calculated from the atomic ratios of carbon to nitrogen. The values of the degree of substitution (DS) were then calculated from the chlorine and sulfur contents where only sulfate groups, in the form of sodium salt, were assumed to be the sulfur-containing groups. Water was assumed to be contained in the samples so as to make the calculated carbon, nitrogen, chlorine and sulfur contents coincide with the experimental values. Calculated values for a sample: DeAc, 36.3%; DS by chlorine, 1.89; DS by sulfate, 0.06; 0.743 mol of water/PRU, C 36.05%; H 4.91%; N 5.78%; Cl 27.58%; S 0.81%. Measured values: C 36.05%; H 4.71%; N 5.78%; Cl 27.58%; S 0.81%.

Gas chromatographic (GC) and gas chromatographic–mass spectrometric (GC–MS) analyses of the hydrolyzates of chlorodeoxychitin samples were carried out as *N,O*-trifluoroacetyl derivatives according to the procedure described in the previous paper (Sakamoto *et al.*, 1994). Chitin samples were hydrolyzed in 35% HCl for 6 h at 80°C for the analysis of constituent saccharides. ^{13}C NMR spectra were recorded at 35°C on JNM-FX90Q (JEOL; 22.53 MHz). For polymer samples, trifluoroacetic acid-*d* was used as a solvent (Sakamoto *et al.*, 1994). A mixture of DCl and D_2O (1:1) was used for the hydrolyzates (external standard, TMS) and the chemical shifts were not corrected for magnetic susceptibility. Angles of rotation were measured with a digital polarimeter DIP-370 (JASCO) using formic acid as a solvent. Reduced viscosities of chitin samples were measured at 40°C and at 0.1 g/dl in LiCl-DMA (5 g/dl).

RESULTS AND DISCUSSION

Furubeppu *et al.* (1991) studied the chlorination of cellulose with sulfuryl chloride under homogeneous conditions in LiCl-DMA in the temperature range −20 to 40°C. At −20°C, the main product was cellulose chlorosulfate, and the nucleophilic substitution of chlorosulfate moieties with chloride ions became remarkable with the rise in reaction temperature. At temperatures above 30°C, the substitution was nearly quantitative and chlorodeoxycellulose samples containing 6-chloro-6-deoxyglucose and 3,6-dichloro-3,6-dideoxyallose units were obtained.

The temperature of the addition of sulfuryl chloride was found to affect the reaction of chitin in LiCl-DMA. Sulfuryl chloride was first added to the chitin solution at −20°C. At this temperature, however, brown gummy materials were sometimes formed and the results were not reproducible in these cases. When the temperature of the addition was lowered to −40°C, yellow homogeneous solutions were obtained in all experiments. Contrary to the case of cellulose, chlorodeoxychitin samples were always obtained in the temperature range −30 to 70°C. However, the products contained small amounts of sulfur and the treatment with sodium iodide (Furubeppu *et al.*, 1991) was not effective in decreasing the sulfur content.

All the isolated chlorodeoxychitin samples were soluble in formic acid. However, samples obtained at 30°C or below were partially insoluble in LiCl–DMA after isolation and those obtained at 40–70°C were almost insoluble. The most probable reason for the insolubility in LiCl–DMA was considered to be the progress of deacetylation during the chlorination observed for the samples obtained at 40°C or higher (see below). Chitosan is soluble in acidic media but insoluble in LiCl–DMA. Another possibility is the formation of crosslinks with sulfate groups. In the calculation of the degree of substitution (DS) by chlorine (DS_{Cl}), however, only the terminal sulfate group (as sodium salt) was assumed as the sulfur-containing group because the samples were treated with a sodium carbonate solution before dialysis against water. Errors due to crosslinking sulfate groups, if present, in the calculation of the DS_{Cl} values are sufficiently small because the sulfur contents of samples are below 1% in most cases.

Extent of chlorination

Preliminary experiments on the effect of the molar ratio of sulfuryl chloride to the repeating unit (PRU) of chitin (reagent ratio, $[SO_2Cl_2]/[PRU]$) at 60°C showed that DS_{Cl} increased with increasing reagent ratio and

reached a maximum at a reagent ratio around 15. The effect of reaction temperature on DS_{Cl} was studied at this reagent ratio. Sulfuryl chloride was added at −40°C and the reactions were carried out for 4 h.

Figure 1 shows that the DS_{Cl} value for the sample obtained at −30°C is 0.65 and samples with DS_{Cl} from 1.0 to 1.4 are obtained in the reactions around 0°C. The DS_{Cl} value increases with the increase in reaction temperature and the sample obtained at 70°C has DS_{Cl} of 1.87. The product yield decreases from 90 to 60% as the reaction temperature rises from −30 to 60°C and drops to 27% at 70°C. The decrease in yield at higher temperatures can be ascribed to the scission of chitin molecular chains (Sakamoto *et al.*, 1994). The values of DS by sulfate group (DS_S) tend to increase slightly with temperature. The values of the degree of deacetylation (DeAc) for products obtained in the reaction at 30°C or lower are close to that of the starting chitin sample (9.7%) while they are around 50% for the products obtained at 40, 60 and 70°C. The reason is not clear for the steep increase in DeAc with the rise in reaction temperature from 30 to 40°C. Chlorodeoxychitin samples with high DS_{Cl} (up to 1.65) and without additional deacetylation are obtained at 30°C or below.

This chlorination is a comparatively fast process in the temperature range studied and DS_{Cl} levels off in 3–4 h. The yield and DeAc show similar trends. At a fixed reagent ratio, the reaction temperature determines the DS_{Cl} and prolongation of reaction time is not effective in increasing the DS_{Cl} of products.

The reduced viscosities of chlorodeoxychitin samples were measured in LiCl–DMA at 40°C. These samples were obtained at temperatures from −30 to 20°C. Their DeAc values were around 10% and were mostly soluble in LiCl-DMA. Figure 2 shows that the reduced viscosities of the soluble parts are about 3 dl/g and independent of the DS_{Cl} values (0.65–1.44). The decrease in reduced viscosity of these samples as compared with that of the purified chitin (6.5 dl/g) is probably due to the replacement of C-6 hydroxyl groups with chlorine atoms and not to the scission of chitin molecular chains during the chlorination (Sakamoto *et al.*, 1994).

Structural studies

The chemical structures of chlorodeoxychitin samples having various DS_{Cl} values were studied with gas chromatographic (GC) and gas chromatographic–mass spectrometric (GC–MS) analyses. ^{13}C NMR spectroscopy was also applied to both the polymers and the hydrolyzates.

Figure 3 shows the GC traces of the hydrolyzates (as *N,O*-trifluoroacetyl derivatives) of chitin and chlorodeoxychitin samples (DS_{Cl}; 1.02, 1.30 and 1.85). The hydrolyzates of chitin and chlorodeoxychitin of DS_{Cl} 1.02 show simple traces. Peaks due to anomers of glucosamine and 6-chloro-6-deoxyglucosamine appear respectively. The structures of the materials of these peaks were confirmed by GC–MS analysis (Sakamoto *et al.*, 1994). For samples with DS_{Cl} of 1.30 and 1.85, the traces became complex and the relative peak areas of saccharides in the hydrolyzates vary according to the hydrolysis conditions. For these hydrolyzates, peaks due to disaccharides were also observed at higher elution temperatures.

Table 1 shows that the material responsible for peak 4 can be ascribed to a pyranose anomer of dichlorodideoxyhexosamine and that for peak 5 to a furanose tautomer. The material of peak 4′ gave essentially the same fragmentation pattern as that of peak 4. The fragmentation patterns show that the C-6 hydroxyl group of the saccharide has been substituted with a chlorine atom. The position of the substitution by the second chlorine atom cannot be determined from the fragmentation pattern but C-3 is the only possible position available for chitin. The material for peak 3 has one chlorine atom attached to a carbon other than C-6, and is tentatively assigned to 3-chloro-3-deoxyhexosamine. This compound is probably an artifact formed from the dichlorodideoxyhexosamine repeating units during hydrolysis. In this study, chitin samples were hydrolyzed

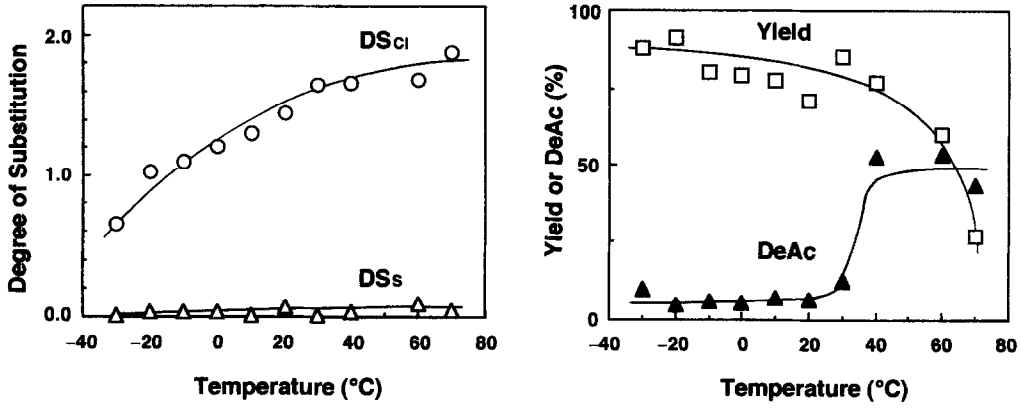

Fig. 1. Effects of reaction temperature on DS_{Cl}, DS_S, yield and DeAc. Reaction conditions, for 4 h at reagent ratio of 15.

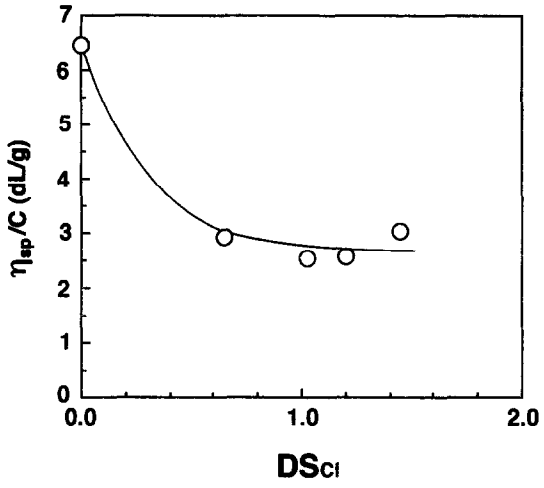

Fig. 2. Reduced viscosity as a function of DS_{Cl}. Viscosities were measured at 40°C in LiCl–DMA.

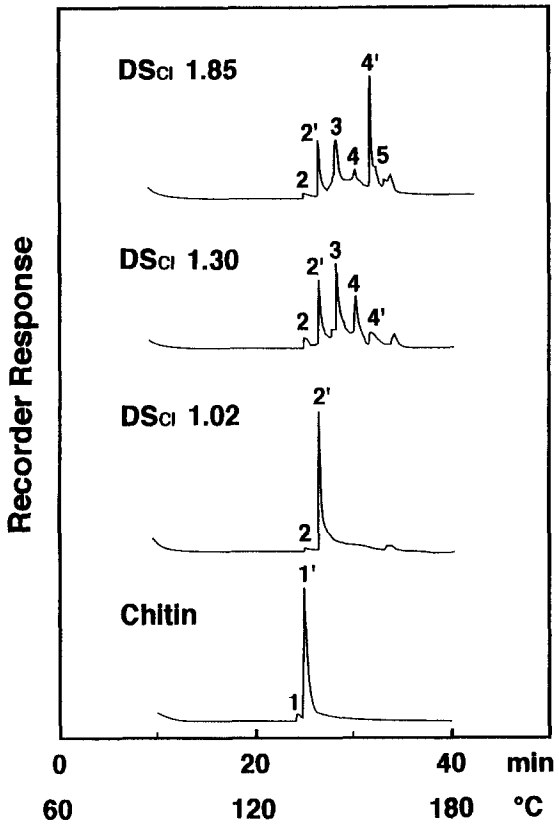

Fig. 3. GC traces for hydrolyzates of chitin and chlorodeoxychitin samples as *N,O*-trifluoroacetyl derivatives: (1&1′), glucosamine(*p*); (2&2′), 6-chloro-6-deoxyglucosamine(*p*); (3), 3-chlorohexosamine(*p*); (4&4′), 3,6-dichloro-3,6-dideoxyhexosamine(*p*); (5), 3,6-dichloro-3,6-dideoxyhexosamine(*f*).

in 35% HCl for 6 h at 80°C. Sakamoto *et al.* (1984) reported that chlorine–carbon bonds were hydrolyzed in 6 N HCl at 110°C.

The NMR spectral analysis confirmed the structures of chlorodeoxychitins revealed by the GC–MS analysis described above. Figure 4 shows ^{13}C NMR spectra (in trifluoroacetic acid-*d*) of the purified chitin and a chlorodeoxychitin sample having DS_{Cl} of 1.85. The absorption of C-6 carbons of chitin at 68 ppm shifts to 45 ppm for the chlorodeoxychitin sample due to the substitution of hydroxyl groups with chlorine atoms (Sakamoto *et al.*, 1994).

In the spectrum of the chlorodeoxychitin sample, several absorptions are present in the region of 60–70 ppm and the absorption at 69 ppm is the strongest among them. This chemical shift value is reasonable for a C-3 carbon, the hydroxyl group of which is substituted with chlorine (Furuhata *et al.*, 1994; Krylova *et al.*, 1981). The number and origin of other small peaks in the 60–70 ppm region are difficult to explain. The DeAc value of this sample is about 50% and therefore nearly equal amounts of *N*-acetylated and deacetylated repeating units are contained. However, this is possibly not the reason for the presence of several absorptions in the region because chemical shifts of pyranose carbons of *N*-acetylglucosamine are close to those of the corresponding carbons of glucosamine hydrochloride (Bock and Pedersen, 1983).

The nucleophilic substitution of hydroxyl (chlorosulfate) groups at C-3 with chloride ions will give repeating units of *allo* configuration. It must be mentioned here that the amount of chloride ions in the reaction medium is much larger than in chitin, and therefore further substitution of C-3 chlorine atoms with chloride ions may occur, which will give repeating units of *gluco* configuration. The C-3 carbons of methyl 3,6-dichloro-3,6-dideoxy-β-D-glucoside and -alloside appeared at 68.55 and 69.07 ppm in D_2O (Furuhata *et al.*, 1994), respectively, and it is difficult to determine the configuration of dichlorodideoxy repeating units based only on the present chemical shift value. However, the substitution of C-3 chlorine atoms with chloride ions in DMA was a substantially slow process in the case of cellulose (Furuhata *et al.*, 1994) and the dichlorodideoxy repeating units are considered to be composed of 3,6-dichloro-3,6-dideoxyallosamine and its *N*-acetylated derivative.

The peaks of the C-1 (102 ppm) and C-2 carbons (59 ppm) of chitin are split into doublets (99 and 103 ppm, and 55 and 57 ppm, respectively) in the spectrum of the chlorodeoxychitin sample. This can be ascribed to the presence of two kinds of repeating units, (*N*-acetyl-)6-chloro-6-deoxyglucosamine and (*N*-acetyl-)3,6-dichloro-3,6-dideoxyhexosamine. The absorption near 93 ppm may be due to C-1 carbons of the reducing ends of chlorodeoxychitins of low molecular weight.

In order to obtain further information on the structures of constituent saccharides, chlorodeoxychitin samples were hydrolyzed in HCl. Figure 5 shows the ^{13}C NMR spectra (in DCl-D_2O) of hydrolyzates of chitin (glucosamine) and two chlorodeoxychitin samples of DS_{Cl} 0.95 and 1.55. The C-1 (91 ppm), C-2 (56 ppm), C-3 (71 ppm) and C-5 (73 ppm) carbons of α-glucosamine appear at higher fields than those of the corresponding

Table 1. Mass fragmentation patterns of peak materials as *N,O*-trifluoroacetyl derivatives

Ion	Peak 3		Peak 4		Peak 5	
	m/z	r.a.	*m/z*	r.a.	*m/z*	r.a.
M^+	581(d)	n.d.	503(t)	0.2	503(t)	n.d.
M–Cl	546(s)	0.03	468(d)	0.2	468(d)	0.8
$M–CF_3COO$	468(d)	0.1	390(t)	1.7	390(t)	1.1
$M–CF_3COO–HCl$	432(s)	n.d.	354(d)	1.5	354(d)	1.6
$M–CF_3COO–CF_3COOH$	354(d)	1.3	276(t)	n.d.	276(t)	n.d.
$M–CF_3COO–HCl$ CF_3COOH	318(s)	7.1	240(d)	17.8	240(d)	21.1
$M–CH_2OCOCF_3$	454(d)	n.d.				
$M–CH_2Cl$			454(d)	0.6		
454–HCl	418(s)	n.d.	418(s)	0.1		
$454–CF_3COOH$	340(d)	7.5	340(d)	1.7		
$418–CF_3COOH$	304(s)	1.4	304(s)	1.1		
$M–CH(OCOCF_3)CH_2Cl$					328(d)	1.5
$328–CF_3COOH$					214(d)	32.2
CF_3	69(s)	100	69(s)	100	69(s)	100

Peak 3: 3-chloro-3-deoxyhexosamine(*p*).
Peak 4: 3,6-dichloro-3,6-dideoxyhexosamine(*p*).
Peak 5: 3,6-dichloro-3,6-dideoxyhexosamine(*f*).
r.a., Relative abundance, %.
n.d., Not detected.
(t), 9:6:1 triplet at *m*, *m*+2 and *m*+4.
(d), 3:1 doublet at *m* and *m*+2.
(s), singlet.

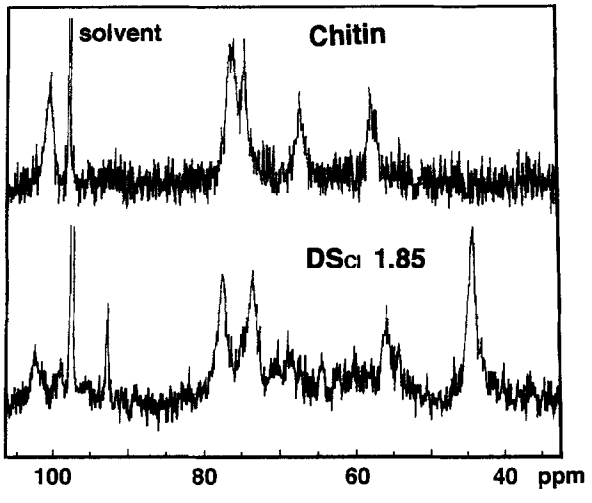

Fig. 4. ^{13}C NMR spectra of chitin and chlorodeoxychitin of DS_{Cl} 1.85. Spectra were measured at 22.53 MHz in trifluoroacetic acid-*d*.

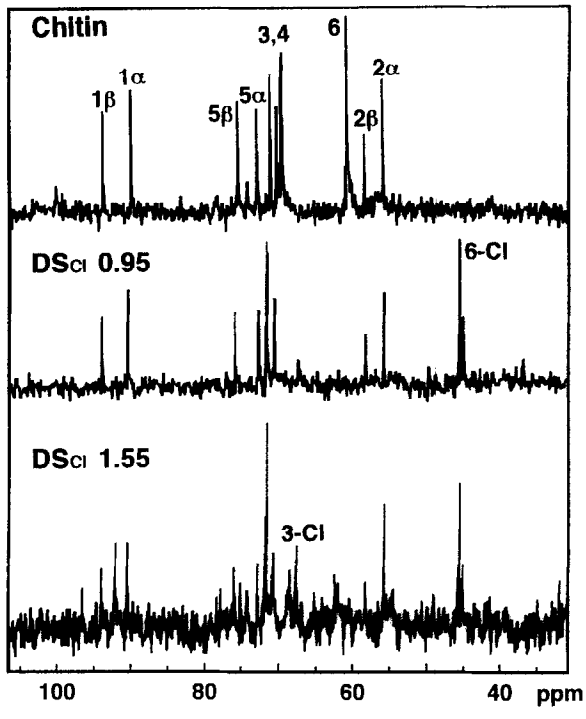

Fig. 5. ^{13}C NMR spectra of hydrolyzates of chitin and chloro deoxychitin samples. Spectra were measured at 22.53 MHz in DCl-D_2O.

β-anomer (94, 58, 72 and 76 ppm, respectively) (Bock and Pedersen, 1983; Walker and Barker, 1978). In the spectrum of the hydrolyzate of the chlorodeoxychitin sample with DS_{Cl} of 0.95, chemical shifts of C-1, C-3 and C-5 carbons are almost the same as those of the anomers of glucosamine. The absorption of C-6 carbons of glucosamine at 62 ppm, on the other hand, is not observed and instead a new peak appears at 46 ppm ascribable to chlorine-bearing C-6 carbons (Furuhata *et al.*, 1994; Krylova *et al.*, 1981). The main compounds contained in this hydrolyzate are therefore anomers of 6-chloro-6-deoxyglucosamine. The absorption of C-4 carbons seems to be shifted from 70 to 72 ppm.

The spectrum of the hydrolyzate of the chlorodeoxychitin sample of DS_{Cl} 1.55 has two groups of absorptions in addition to those observed in the above-

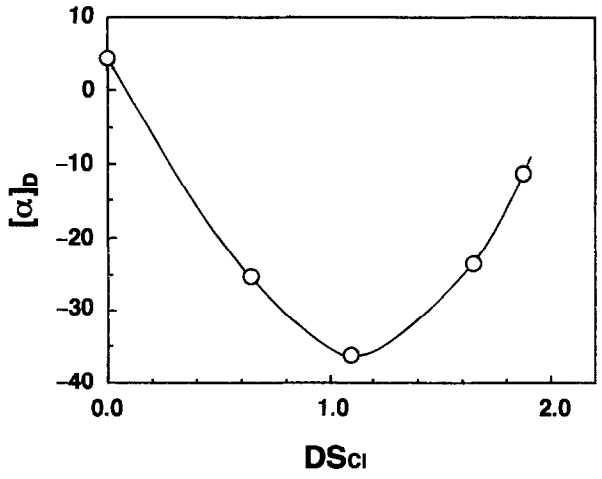

Fig. 6. Specific rotation as a function of DS_{Cl}. Angles of rotation were measured in formic acid.

mentioned spectrum. One of the pair of peaks in C-1 carbon region (92 and 97 ppm) and the other is the two absorptions at 68 and 69 ppm. The former peaks can be ascribed to C-1 carbons of dichlorohexosamine anomers and the latter peaks to the chlorine-bearing C-3 carbons (Furuhata *et al.*, 1994; Krylova *et al.*, 1981). The main compounds contained in this hydrolyzate are therefore anomers of 6-chloro-6-deoxyglucosamine and those of 3,6-dichloro-3,6-dideoxyhexosamine. A weak absorption at 62 ppm may be due to hydroxyl-bearing C-6 carbons formed from chlorine-bearing carbons during hydrolysis as revealed by the GC and GC–MS analyses described above.

Figure 6 shows the values of specific rotation of chlorodeoxychitin samples in formic acid. The DeAc values for samples are around 10% except for the sample of the highest DS_{Cl}, whose DeAc is 46.6%. The specific rotation becomes levorotatory and the change is fairly linear against DS_{Cl} up to 1. On the contrary, the amount of dextrorotatory component in the sample increases with increasing DS_{Cl} when the sample DS_{Cl} exceeds 1. This is in agreement with the anticipated change in configuration of the repeating units in the chlorodeoxychitin samples, from *N*-acetyl-6-chloro-6-deoxyglucosamine to *N*-acetyl-3,6-dichloro-3,6-dideoxy-allosamine.

In the present study, both C-6 and C-3 hydroxyl groups of chitin could be substituted with chlorine atoms. We expect that these highly chlorinated chitin samples will be useful for the introduction of highly functional groups into chitin.

REFERENCES

Bock, K. and Pedersen, C. (1983) *Adv. Carbohydr. Chem. Biochem.* **41**, 27–66.

Classon, B., Garegg, P.J. and Samuelsson, B. (1981) *Can. J. Chem.* **59**, 339–343.

Furubeppu, S., Kondo, T. and Ishizu, A. (1991) *Sen'i Gakkaishi* **47**, 592–597.

Furuhata, K., Chang, H.-S., Aoki, N. and Sakamoto, M. (1992) *Carbohydr. Res.* **230**, 151–164.

Furuhata, K., Aoki, N., Suzuki, S., Arai, N., Sakamoto, M., Saegusa, Y. and Nakamura, S. (1994) *Carbohydr. Res.* **258**, 169–178.

Furuhata, K., Aoki, N., Suzuki, S., Sakamoto, M., Saegusa, Y. and Nakamura, S. (1995) *Carbohydr. Polym.* **26**, 25–29.

Hodosi, G., Podányi, B. and Kuszmann, J. (1992) *Carbohydr. Res.* **230**, 327–342.

Krylova, R.G., Usov, A.I. and Shashkov, A.S. (1981) *Soviet J. Bioorg. Chem.* **7**, 871–877.

Sakamoto, M., Kohara, N., Wakabayashi, M., Nishimoto, M. and Nakayama, F. (1984) *Sen'i Gakkaishi* **40**, 113–121.

Sakamoto, M., Tseng, H. and Furuhata, K. (1994) *Carbohydr. Res.* **265**, 271–280.

Tokura, S. (1983) *Kobunshi Kako* **23**, 162–170.

Tseng, H., Furuhata, K. and Sakamoto, M. (1995) *Carbohydr. Res.* **270**, 149–161.

Tseng, H., Lee, R.-S., Furuhata, K. and Sakamoto, M. (1995) *Sen'i Gakkaishi* **51**, 540–543.

Walker, T.E. and Barker, R. (1978) *Carbohydr. Res.* **64**, 266–270.