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Novel *N*-unsaturated fatty acyl and *N*-trimethylacetyl derivatives of chitosan

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Novel *N*-oleoyl (d.s. 0·93), *N*-linolenoyl (d.s. 0·93), *N*-elaidoyl (d.s. 0·88), *N*-erucoyl (d.s. 0·87) and seven *N*-trimethylacetyl (d.s. 0·16–0·85) derivatives of chitosan were isolated in 54–91% yields as a gel or a precipitate from the corresponding carboxylic anhydride. These derivatives were insoluble in common organic solvents, except for partially *N*-trimethylacetyl derivatives (d.s. 0·30–0·54) which were soluble in water. *N*-Trimethylacetyl derivative (d.s. 0·65) was isolated in 83% yield, and was insoluble in water. Their chemical structures were determined with reference to the corresponding crystalline derivatives of D-glucosamine.

INTRODUCTION

Chitosan, (1→4)-β-D-glucosaminan, has a chemically definite linear structure, and has one reactive amino group and two hydroxyl groups in the repeating moiety. Chemical modification of these groups gives rise to novel molecular functions involving gelation (Zhang *et al.*, 1994), water solubility (Sannan *et al.*, 1975, 1976; Hirano *et al.*, 1991), blood compatibility (Hirano & Noishiki, 1985), anticoagulant activity (Hirano *et al.*, 1985), and antitumor activity (Ouchi *et al.*, 1989). A series of *N*-saturated higher fatty acyl derivatives (d.s. 0·78–1·00) of chitosan (Hirano *et al.*, 1976) and a microbial (1→4)-α-D-galactosaminan (Zhang *et al.*, 1994) have been prepared, but no report has dealt with *N*-unsaturated higher fatty acyl and bulky *N*-trimethylacetyl derivatives of chitosan. The *N*-higher fatty acyl groups on the β-D-glucosaminyl moiety in lipid A are a bioactive part of the endotoxins of Gram-negative bacteria (Fasman, 1975). The *N*-higher fatty acyl derivatives of polysaccharides are utilized as a coating material for the surface of liposomes. This is because of their stability and for targeting to specific cells and organs (Takeda *et al.*, 1984).

We now report the preparation, structural analysis, and some unique properties of *N*-unsaturated higher fatty acyl and *N*-trimethylacetyl derivatives of chitosan, and *N*-trimethylacetyl galactosaminan, in comparison with those of D-glucosamine. The derivatives studied are shown in Fig. 1.

EXPERIMENTAL

Materials

Crab shell chitosan (d.s. 0·01 for NAc) was a product of the Katakurachikkarin Company Ltd, Tokyo. A microbial (1→4)α-D-galactosaminan was a product of Higeta Shoyu Ltd, Chiba (Takeda & Kadowaki, 1985). *N*-Stearoylchitosan was prepared as reported (Hirano *et al.*, 1976). D-Glucosamine hydrochloride was isolated by acid hydrolysis from crab shell chitin (Purchase & Braun, 1955). Trimetehylacetic anhydride was purchased from Aldrich Chemical Company, Milwaukee, and unsaturated higher fatty acid anhydrides from Sigma, St Louis.

Methods

^{13}C CPMAS NMR spectra were recorded on a Chemagnetics CMX 360 NMR spectrometer (Chemagnetics Co., Ltd, Fort Collins), ^{13}C NMR spectra (D_2O) on a Jeol JNM-GX 270 FTNMR spectrometer (Jeol Co., Ltd, Tokyo), FTIR spectra (KBr) on a Jasco FTIR 5300 spectrometer (Jasco Co., Ltd, Tokyo), and specific rotations on a Horiba SEPA-200 autopolarimeter (Horiba Co., Ltd, Kyoto). Elemental analyses were performed at the Micro-analytical Center of Kyoto University, Kyoto. D.s. values for *N*-acyl groups were calculated from the elemental analysis data.

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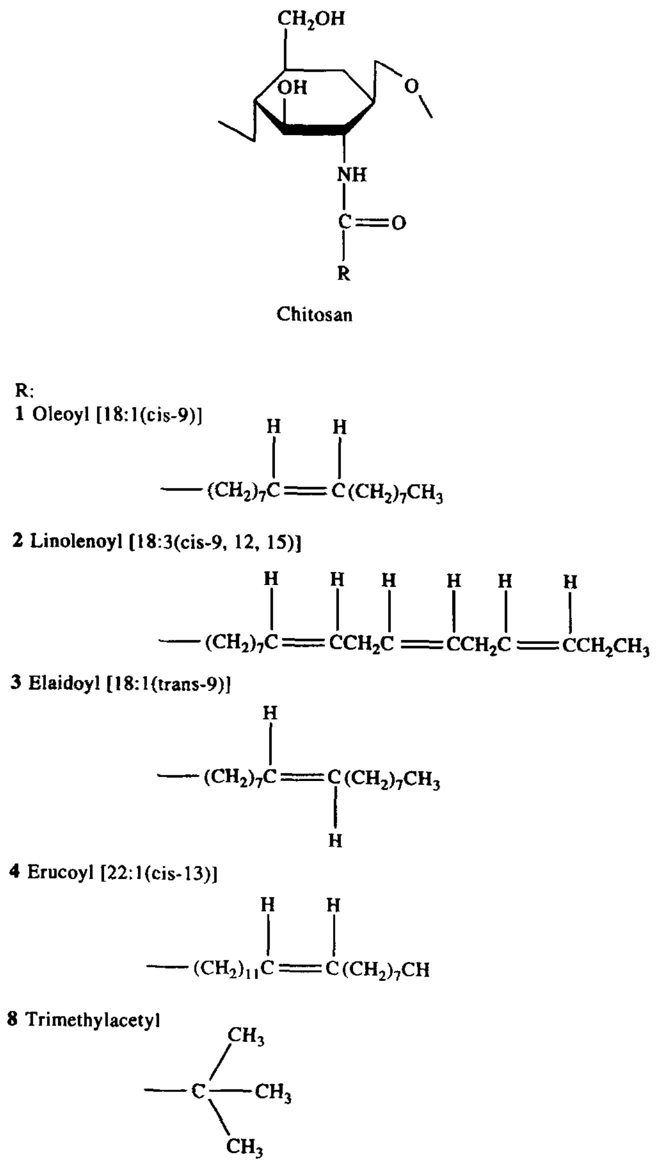

Fig. 1

N-Unsaturated higher fatty acyl derivatives (1–4) of chitosan

Chitosan (0·16 g) was dissolved in aqueous 2% acetic acid (10–15 ml), and the solution was diluted with methanol (30 ml) (Hirano *et al.*, 1976). Each (3 mol/GlcN) of elaidic, oleic, erucoic and linolenic anhydrides was added with stirring. The mixture was heated at about 60°C for a few seconds to give a white precipitate or a transparent gel was produced at 70–87% yields. ν_{max} (KBr) cm^{-1}: 1651–1655 and 1550–1554 (C=O and NH of *N*-acyl).

N-Unsaturated fatty acyl derivatives (5–7) of D-glucosamine

To a solution of D-glucosamine in methanol prepared from its hydrochloride (0·3 g) (Inouye *et al.*, 1956), each (3 mol/GlcN) of elaidic, oleic, erucic, anhydrides was added, and the mixture was heated for a few minutes at approximately 60°C in a water bath to afford a clear solution. The solution was kept in a refrigerator overnight to afford the corresponding crystalline *N*-acyl derivatives in 52–72% yields (Table 3). ν_{max} (KBr) cm^{-1}: 1641–1643 and 1552–1558 (C=O) and NH of *N*-acyl. These compounds were soluble in pyridine, but insoluble in water. **5**, $[\alpha]^{20}_D$ +53°→54° (*c* 1·0, pyridine); **6**, $[\alpha]^{20}_D$ +54°→68° (*c* 1·0, pyridine).

N-Trimethylacetyl derivatives of chitosan (8) and galactosaminan (9)

Chitosan (0·16 g) was dissolved in aqueous 2% acetic acid (10–15 ml), and the solution was diluted with methanol (30 ml). Several molar ratios of trimethylacetic anhydride per GlcN or GalN were added with stirring, and the solution mixture was kept at room temperature overnight to give a solution or a gel. The gel was ground into small particles by a homogenizer. The product was dialysed against running water for 1 day, and was concentrated *in vacuo* into an approximately 100 ml volume. After adjusting to pH 9 with aqueous 2% NaOH, three volumes of ethanol were added to give a precipitate which was collected by filtration, washed several times with ethanol, and dried to give a series of partially *N*-trimethylacetyl derivatives (**8**) in 54–91% yields. ν_{max} (KBr) cm^{-1}: 1637–1649 and 1539–1545 (C=O and NH of *N*-trimethylacetyl).

N-Trimethylacetyl galactosaminan was obtained in 83% yield, and was insoluble in common solvents including water. ν_{max} (KBr) cm^{-1}: 1635 and 1539 (C=O and NH of *N*-trimethylacetyl). Anal. Calc. for $[C_6H_{10}O_4N(C_5H_9O)_{0·65}(H)_{0·35}\cdot 1·32H_2O]n$: C, 46·50; H, 7·62; N, 5·86. Found: C, 46·64; H, 7·27; N, 5·93.

N-Trimethylacetyl D-glucosamine (10)

D-Glucosamine HCl (1·0 g) was treated with sodium metal (0·1 g) in anhydrous methanol (10 ml), and the mixture was filtered (Inouye *et al.*, 1956). To the clear filtrate solution, trimethylacetic anhydride (1·2 g, 1·2 mol equivalent to GlcN) was added, and the mixture was maintained at room temperature overnight to give a crystalline product, which was recrystallized from aqueous ethanol, 62% yield, m.p. 184–186°, $[\alpha]^{20}_D$ +50°→+160° (*c* 1, water). The compound was soluble in water, pyridine, aqueous acidic and alkaline solutions, but insoluble in ethanol, acetone and DMSO. ν_{max} (KBr) cm^{-1}: 1645 and 1535 (C=O and NH of *N*-trimethylacetyl). ^{1}H NMR data (D_2O): δ 5·18 (d, 0·5 proton, H-1, $J_{1,2}$ 0·6 Hz), 4·81 (0·5 proton, H-1, $J_{1,2}$ 7·5 Hz), 0·39–0·37 (6 protons, H-2, H-3, H-4, H-5, H-6, H-6′), 1·19 (9 protons, $3\times CH_3$). Anal. Calc. for $C_{11}H_{21}O_6N$: C, 50·20; H, 8·27; N, 5·23. Found: C, 50·14; H, 8·23; N, 5·20.

RESULTS AND DISCUSSION

Reaction of chitosan with a corresponding unsaturated higher fatty acid anhydride (3 mol/GlcN), *N*-oleoyl (**1**) and *N*-linolenoyl (**2**) gave derivatives (d.s. 0·93) at 80–81% yield as a gel, and *N*-elaidoyl (**3**) and *N*-erucoyl (**4**) derivatives (d.s. 0·87–0·88) at 70–87% yields as an amorphous precipitate (Table 1). Under the same conditions, *N*-higher saturated fatty acyl derivatives (> C14) of chitosan were produced as an amorphous precipitate (Hirano *et al.*, 1976). The formation of a gel or precipitate was dependent on both the d.s. and chemical structure of the *N*-acyl group in *N*-acyl derivatives of chitosan (Hirano & Tamaguchi, 1976).

N-Elaidoyl (**5**, m.p. 192–194°), *N*-oleoyl (**6**, m.p. 194–196°), and *N*-erucoyl (**7**, m.p. 182–186°) derivatives of D-glucosamine were obtained as crystals of an anomeric mixture at 52–72% yields (Table 2). No liquid *N*-acyl product was produced, although these unsaturated higher fatty acids and anhydrides are liquid at room temperature.

Chitosan was treated with trimethylacetic anhydride (0·5–30 mol/GlcN) to afford a series of partially *N*-trimethylacetyl derivatives (**8**) at 54–91% yields (Table 3). A gel formed in **8** at a d.s. higher than 0·67 for *N*-trimethylacetyl in reactions with an excess amount of trimethylacetic anhydride (20–30 mol/GlcN). *N*-Trimethylacetyl galactosaminan (**9**, d.s. 0·65) was also obtained as an amorphous precipitate at 83% yield. *N*-Trimethylacetyl β-D-glucosamine (**10**) was isolated as a crystal at 62% yield. The anomeric configuration was confirmed by a mutarotation, $[\alpha]_D^{20}$ +50°→+160° (*c* 1, water), and by a wide $J_{1,2}$ coupling of H-1 in ^{1}H-NMR spectra. On the other hand, a small value of mutarotation in **5** {$[\alpha]_D^{20}$ +53°→54° (*c* 1·0, pyridine)}, and in **6** {+54→+68° (*c* 1, pyridine)} supported their anomeric mixture. These data strongly indicate that the anomeric configuration is strongly affected by the bulky *N*-trimethylacetyl group at C2, but is only slightly affected by the *N*-higher fatty acyl groups. Chemical structures of these compounds were also confirmed by the elemental analyses.

The solubility of *N*-acyl derivatives of chitosan in water is dependent mainly on d.s., distribution, and structure of *N*-acyl groups (Sannan *et al.*, 1975, 1976;

Table 1. Data for four *N*-unsaturated fatty acyl derivatives of chitosan

Compound	Yield (%)	d.s. for *N*-Acyl	Formula[a]	Calc.			Found		
				C	H	N	C	H	N
1	80	0·93	$[R(C_{18}H_{32}O)_{0{\cdot}93}(H)_{0{\cdot}07} \cdot 0{\cdot}53H_2O]_n$	65·73	9·85	3·37	65·69	10·13	3·31
2	81	0·93	$[R(C_{18}H_{29}O)_{0{\cdot}93}(H)_{0{\cdot}07} \cdot 0{\cdot}78H_2O]_n$	65·61	9·06	3·36	65·37	9·57	3·37
3	87	0·88	$[R(C_{18}H_{32}O)_{0{\cdot}88}(H)_{0{\cdot}12} \cdot 0{\cdot}40H_2O]_n$	65·43	9·98	3·50	65·28	10·19	3·44
4	70	0·87	$[R(C_{22}H_{41}O)_{0{\cdot}87}(H)_{0{\cdot}13} \cdot 0{\cdot}50H_2O]_n$	65·32	10·18	3·53	65·18	10·48	3·53

[a] R: $C_6H_{10}NO_4$.

Table 2. Data for some *N*-unsaturated fatty acyl derivatives of D-glucosamine

Compound	Yield (%)	M.p. (°)	Formula	Calc.			Found		
				C	H	N	C	H	N
5	52	192–194	$C_{24}H_{45}O_6N$	64·98	10·23	3·16	64·77	10·49	3·02
6	69	194–196	$C_{24}H_{45}O_6N$	64·98	10·23	3·16	64·72	10·35	3·11
7	72	182–186	$C_{28}H_{53}O_6N$	67·30	10·69	2·80	66·96	10·73	2·62

Table 3. Data for some *N*-trimethylacetyl derivatives (8) of chitosan

Trimethylacetic anhydride (mol/GlcN)	d.s. for *N*-Acyl	Yield (%)	Formula[a]	Calc.			Found		
				C	H	N	C	H	N
0·5	0·16	54	$[R(C_5H_9O)_{0{\cdot}16}(H)_{0{\cdot}84} \cdot 0{\cdot}59H_2O]_n$	44·29	6·85	7·60	44·22	7·14	7·59
0·65	0·20	58	$[R(C_5H_9O)_{0{\cdot}20}(H)_{0{\cdot}80} \cdot 0{\cdot}86H_2O]_n$	43·61	7·03	7·28	43·43	7·31	7·27
1·0	0·30	67	$[R(C_5H_9O)_{0{\cdot}30}(H)_{0{\cdot}70} \cdot 0{\cdot}75H_2O]_n$	45·22	7·14	7·04	45·02	7·49	7·02
3·0	0·54[b]	79	$[R(C_5H_9O)_{0{\cdot}54}(H)_{0{\cdot}46} \cdot 0{\cdot}95H_2O]_n$	46·82	7·51	6·28	46·96	7·23	6·33
10	0·67	82	$[R(C_5H_9O)_{0{\cdot}67}(H)_{0{\cdot}33} \cdot 0{\cdot}57H_2O]_n$	48·19	7·51	6·01	48·16	7·69	5·99
20	0·85	91	$[R(C_5H_9O)_{0{\cdot}85}(H)_{0{\cdot}15} \cdot 1{\cdot}85H_2O]_n$	46·75	7·22	5·32	46·64	7·14	5·28
30	0·80	88	$[R(C_5H_9O)_{0{\cdot}80}(H)_{0{\cdot}20} \cdot 1{\cdot}67H_2O]_n$	46·88	7·20	5·47	46·78	7·30	5·52

[a] R: $C_6H_{10}NO_4$.

[b] $[\alpha]_D^{20}$ − 21° (*c* 1·0, aqueous 2% acetic acid).

Table 4. Solubility of the partially *N*-trimethylacetyl derivatives (8) of chitosan

d.s. for *N*-acetyl	Water	Aqueous 1% NaOH	Aqueous 2% acetic acid
0·0	i	i	s
0·16	i	i	s
0·20	i	ss	s
0·30	ss	s	s
0·54	s	s	s
0·67	ss	i	ss
0·80	i	i	i
0·85	i	i	i

s, soluble; ss, slightly soluble; i, insoluble.

Aiba, 1991). Compound **8** (d.s. about 0·54) was soluble in water, aqueous 1% NaOH, and aqueous 2% acetic acid (Table 4), in agreement with the solubility in water of partially *N*-acetyl derivatives (d.s. *c* 0·5) (Sannan *et al.*, 1975, 1976). However, **8** (d.s. > 0·8 and < 0·2 for *N*-trimethylacetyl) was insoluble in water. Compounds **5–7** were soluble in pyridine but insoluble in water, chloroform, and ethanol.

^{13}C CPMAS NMR spectral analysis is a useful tool for the analysis of conformation and chemical structure in the solid state (Gerstein, 1985; Ando, 1994). ^{13}C signals in the present study were conventionally assigned in comparison to those of water-soluble chitin oligosaccharides, D-glucosamine hydrochloride, and *N*-acetyl D-glucosamine (Saito *et al.*, 1981*a*, 1981*b*). As shown in Table 5, ^{13}C signals for C1 (104·1 and 104 ppm) and C4 (83·4 and 84 ppm) in **8** and *N*-acetylchitosan appeared in lower magnetic fields than those (92·2–98·0 and 72·0–73·7 ppm) in **5**, **10** and *N*-acetyl D-glucosamine due to the 1→4 glycosidic linkage (Saito *et al.*, 1981*b*). ^{13}C signals (28·2–29·3 ppm) for CH_3 in **8** and **10** were in a slightly lower field than those (23·0–24·6 ppm) in *N*-acetylchitosan and *N*-acetyl D-glucosamine, and ^{13}C signal for the terminal CH_3 of *N*-elaidoyl group in **5** appeared at 14·3 ppm. ^{13}C signal for the terminal CH_3 of *N*-elaidoyl group in **5** appeared at 14·3 ppm. ^{13}C signals (181·8–186 ppm) for C=O of *N*-trimethylacetyl in **8** and **10** were in a lower field than those (173·8–175·3 ppm) in **5**, **6**, *N*-acetyl D-glucosamine and *N*-acetylchitosan. ^{13}C signals for C=O of *N*-acyl groups split into two signals at 173·0–177·4 ppm for *β*-D-configuration and 172·2–173·9 ppm for *α*-D-configuration in **5**, **6**, and *N*-acetyl D-glucosamine, although the ^{13}C signal was apparently one signal in **8**, **10**, and *N*-acetylchitosan, and was apparently one signal in **5**, **6**, **8**, **10** and *N*-acetylchitosan. Two ^{13}C signals for C=O (177·4 and 177·2 ppm for *β*- and *α*-D-configurations) were also detected in an anomeric mixture of *N*-acetyl D-glucosamine (Table 5). ^{13}C signals for C=O and C1–C5 in the *α*-D-configuration of **5**, **6**, **10**, and *N*-acetyl D-glucosamine in both D_2O and pyridine-d_6 appeared in a slightly higher field with a stronger signal than those in the *β*-D-configuration. However, ^{13}C signal for C6 of *α*-D-

Table 5. **^{13}C NMR data for *N*-trimethylacetyl** (8) **and *N*-acetyl derivatives of chitosan with reference to those for *N*-**elaidoyl (5), ***N*-oleoyl** (6), ***N*-trimethylacetyl** (10) **and *N*-acetyl derivatives of D-glucosamine**

Compound		10	5	6	*N*-Acetyl-D-glucosamine		8	*N*-acetyl-chitosan
Solvent		A	B	B	A	B	C	C
C1	*β*	97·6	98·0	98·0	97·6	97·0	104·1	104
	α	93·5	92·8	92·8	93·5	92·2		
C2	*β*	59·4	59·2	59·8	59·4	58·8	59·9	56
	α	56·8	56·1	56·3	56·8	55·8		
C3	*β*	76·3	76·8	77·0	76·6	75·8	75·9	76
	α	73·2	73·8	73·6	73·4	72·5		
C4	*β*	72·9	73·6	73·7	72·8	72·3	83·4	84
	α	72·8	73·2	73·2	72·6	72·0		
C5	*β*	78·6	78·7	78·5	78·7	77·9	78·0	76
	α	74·3	73·8	73·8	74·3	73·3		
C6	*β*	63·5	63·1	63·0	63·5	62·4	59·9	61
	α	63·4	63·3	63·3	63·3	62·5		
C=O	*β*	186	175·3	175·3	177·4	173·0	181·8	174
	α		173·9	173·8	177·2	172·2		
$C(Me_3)_3$		41·5					39·8	
CH_3	*β*	29·3	14·3	14·3	24·4	23·2	28·2	23
	α				24·6	23·0		
-C=C-		130·8	130·2					
$-CH_2-$		22·8–36·8	22·9–36·8					

A, D_2O; B, pyridine-d_6; C, a solid state.

Table 6. Absorptions for the C=O of *N*-acyl group with respect to the anomeric configuration in FTIR spectra of some *N*-acyl derivatives of chitosan and D-glucosamine

N-Acyl	C=O cm^{-1}		
	Chitosan	D-Glucosamine	
	β	β	α
Acetyl	1660		1628[a]
Trimethylacetyl	1649	1647	
Oleoyl	1651	1647	1626
Linolenoyl	1651	n.d.[b]	
Elaidoyl	1651	1643	1626
Erucoyl	1655	1641	1624
Stearoyl	1651	1643	1624

[a] N-Acetyl α-D-glucosamine, m.p. 204–205°, $[\alpha]_D^{20}$ + 56→ + 40° (*c*. 1·0, water).

[b] Not determined.

configuration appeared in a lower field in pyridine-d_6, but in a higher field in D_2O with stronger signal than those in the β-D-configuration. In FTIR spectra, one absorption band at 1649–1660 cm^{-1} for C=O of *N*-acyl appeared in **1**–**4**, **8**, and *N*-acetyl and *N*-stearoyl derivatives of chitosan. On the other hand, two absorption bands at 1641–1647 and 1624–1626 cm^{-1} appeared in **5**–**7** and *N*-stearoyl D-glucosamine (Table 6). Only one absorption band at 1628 cm^{-1} appeared in *N*-acetyl α-D-glucosamine, and only one absorption band at 1647 cm^{-1} in that of *N*-trimethylacetate β-D-glucosamine. Each of the anomeric mixtures of **5**–**7** is supported by not only the splitting of ^{13}C signal and the two IR absorption bands for C=O, but also the relatively wide range of m.p. and small values of their mutarotation (Table 2).

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