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Note

Selective *N*-acylation of chitosan*

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Chitosan[2] [(1→4)-2-amino-2-deoxy-β-D-glucan] is found in some microorganisms[3] and is readily prepared from chitin by *N*-deacetylation with alkali[4].

Drastic procedures are known for the *N*-acylation of chitosan with carboxylic anhydrides at elevated temperature[5], and with acetic anhydride in the presence of hydrogen chloride or conc. hydrochloric acid[6]. A mild procedure[7,8] involving acetic anhydride in aqueous methanol (neutral or basic) was used for the selective *N*-acetylation of some oligosaccharides of chitosan[9]. However, chitosan is insoluble in these solvents and in other solvents commonly used for the acylation of amino sugars and glycosaminoglycans.

We now report a mild procedure for the selective *N*-acylation of chitosan by treatment of a solution in aqueous, methanolic acetic acid with carboxylic anhydrides at room temperatures.

N-Acylchitosans are of interest, as they effect the selective aggregation of some cancer cells[10].

Fig. 1 shows the rate of acylation of 2-amino-2-deoxy-D-glucose with acetic anhydride in the solvent systems aqueous, methanolic acetic acid, 10% aqueous acetic acid, and 10% aqueous hydrochloric acid. Acylation was very slow in the last solvent because the amino group is extensively protonated. 2-Acetamido-2-deoxy-D-glucose was isolated after using the first solvent system and 2 mol. of acetic anhydride.

Chitosan is soluble in aqueous solutions of formic, acetic, propionic, butyric acids, *etc.*, in which chitosan exists partially as the quaternary ammonium carboxylates[11]. The addition, severally, of carboxylic anhydrides (2–3 mol. per hexosaminide residue) to solutions of chitosan in aqueous, methanolic acetic acid gave the series of *N*-acylchitosans shown in Table I, in yields of 77–96%. The use of aqueous acid as solvent together with 20–40 mol. of carboxylic anhydride per hexosaminide residue gave the *N*-acylchitosans shown in Table II.

All of the dried *N*-acylchitosans were gelatinous and hygroscopic, insoluble in cold and boiling water, 50% formic acid, 10% acetic acid, formic acid, acetic acid, acidic methanol, inorganic acids and alkalis, methyl sulphoxide, formamide, 50%

*For a preliminary report, see Ref. 1.

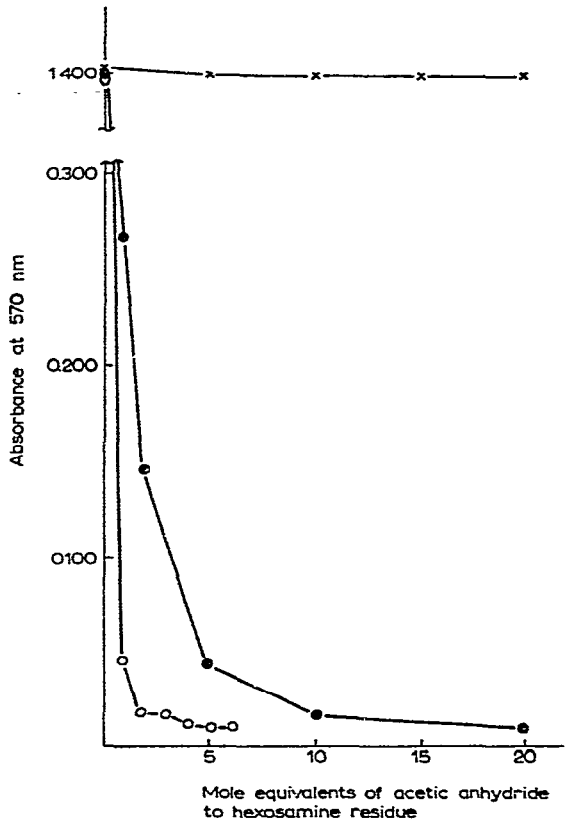

Fig. 1. Reactions of acetic anhydride (1–20 mol.) with 2-amino-2-deoxy-D-glucose (18 mg) in aqueous, acidic solutions (2 ml); aqueous, methanolic acetic acid (—○—), 10% acetic acid (—●—), and 10% aqueous hydrochloric acid (—×—). After keeping at 4° for 24 h, 5 ml of distilled water was added, and to an aliquot (0.05 ml) 1 ml of ninhydrin reagent [2% in 4M acetate buffer (pH 5.51)] was added. The mixture was heated on a boiling water-bath for 15 min and then diluted with 10 ml of distilled water, and the absorbance at 570 nm was determined.

aqueous resorcinol, alcohols, and acetone. However, the *N*-acetyl, *N*-propionyl, and *N*-butyryl derivatives were soluble in formic acid.

The *N*-acylchitosans in Table I showed strong i.r. absorptions for fatty acid at ~2900 cm^{-1} (C–H), and for *N*-acyl groups at ~1650 (C=O) and ~1540 cm^{-1} (N–H), but no absorption for *O*-acyl groups [~1750 (C=O) and ~1240 cm^{-1} (C–O)]. The i.r. spectrum of *N*-acetylchitosan is identical with that of chitin, but differs distinctly from those of chitosan [1610 cm^{-1} (NH_2)] and chitosan hydroacetate [1400, 1550 cm^{-1} (quaternary ammonium acetate)][11]. The *N*-acylchitosans show a degree of substitution (d.s.) of 0.82–1.00 per hexosaminide residue. The d.s. values are based on the ratio of the signal intensities for *N*-acyl protons and methine and methylene protons of the sugar in the n.m.r. spectra, and on the elemental analyses (Table I).

TABLE I

N-ACYLCHITOSANS PREPARED BY THE SELECTIVE *N*-ACYLATION OF CHITOSAN WITH CARBOXYLIC ANHYDRIDES IN AQUEOUS, METHANOLIC ACETIC ACID

N-*Acyl group*	*Yield* (%)	*D.s.*[a]	*Formula*	*Calc.*			*Found*		
				C	*H*	*N*	*C*	*H*	*N*
Acetyl[b]	92	1.00	$[C_8H_{13}NO_5]_n$	47.29	6.45	6.89	47.51	6.28	6.68
Propionyl	92	1.00	$[C_9H_{15}NO_5]_n$	49.76	6.96	6.45	49.75	6.90	6.45
Butyryl	92	1.00	$[C_{10}H_{17}NO_5]_n$	51.94	7.41	6.06	51.91	7.51	6.05
Hexanoyl	96	1.00	$[C_{12}H_{21}NO_5]_n$	55.58	8.16	5.40	55.27	8.39	5.61
Octanoyl	92	0.83	$[C_6H_{10}NO_4(H)_{0.17}(C_8H_{15}O)_{0.83}]_n$	57.08	8.59	5.27	57.32	8.27	5.27
Decanoyl	93	1.00	$[C_{16}H_{29}NO_5]_n$	60.93	9.27	4.44	61.19	9.04	4.77
Lauroyl	88	0.92	$[C_6H_{10}NO_4(H)_{0.08}(C_{12}H_{23}O)_{0.92}]_n$	62.22	9.59	4.26	62.36	9.51	4.27
Myristoyl	77	0.87	$[C_6H_{10}NO_4(H)_{0.13}(C_{14}H_{27}O)_{0.87}]_n$	63.43	9.87	4.07	63.44	9.83	4.13
Palmitoyl	87	0.82	$[C_6H_{10}NO_4(H)_{0.18}(C_{16}H_{31}O)_{0.82}]_n$	64.37	10.08	3.93	64.38	10.14	3.95
Stearoyl	80	0.83	$[C_6H_{10}NO_4(H)_{0.17}(C_{18}H_{35}O)_{0.83}]_n$	65.77	10.36	3.66	65.68	10.27	3.66
Benzoyl	90	0.82	$[C_6H_{10}NO_4(H)_{0.18}(C_7H_5O)_{0.82}]_n$	57.19	5.85	5.68	57.08	5.84	5.67

[a]Degree of substitution. The value is based on the elemental analyses as shown in the formula. [b]$[\alpha]_D^{15}$ −4° (*c* 0.96, formic acid).

TABLE II

ACYLCHITOSANS PREPARED BY ACYLATION OF CHITOSAN (0.1 g) WITH CARBOXYLIC ANHYDRIDES IN AQUEOUS CARBOXYLIC ACIDS

Acyl group	*Yield* (%)	$[\alpha]_D^{15}$ (*formic acid*) (*degrees*)	*D.s.*	*Formula*	*Calc.*			*Found*		
					C	*H*	*N*	*C*	*H*	*N*
Acetyl[a]	93	−1.4 (*c* 1.12)	1.39	$[C_8H_{12}NO_5(H)_{0.61}(C_2H_3O)_{0.39}]_n$	48.01	6.34	6.38	47.93	6.03	6.28
Acetyl[b]	100	−1.3 (*c* 1.00)	1.53	$[C_8H_{12}NO_5(H)_{0.47}(C_2H_3O)_{0.53}]_n$	48.25	6.30	6.21	48.25	6.30	6.20
Propionyl[c]	104	−2.8 (*c* 0.72)	1.30	$[C_9H_{14}NO_5(H)_{0.70}(C_3H_5O)_{0.30}]_n$	50.80	6.99	5.99	50.78	7.20	6.09
Propionyl[d]	110	n.d.[f]	1.25	$[C_9H_{14}NO_5(H)_{0.75}(C_3H_5O)_{0.25}]_n$	50.63	6.99	6.06	50.59	7.09	5.96
Butyryl[e]	103	−1.1 (*c* 0.94)	1.10	$[C_{10}H_{16}NO_5(H)_{0.90}(C_4H_7O)_{0.10}]_n$	52.41	7.46	5.88	52.38	7.40	5.85

[a]10% Acetic acid + acetic anhydride (2.5 ml). [b]50% Formic acid + acetic anhydride (2.5 ml). [c]10% Propionic acid + propionic anhydride (3.4 ml). [d]10% Acetic acid + propionic anhydride (2.5 ml). [e]10% Butyric acid + butyric anhydride (2.5 ml). [f]Not determined.

However, the presence of both *N*- and *O*-acyl groups is evident in acetyl-, propionyl-, and butyryl-chitosans prepared using 10% acetic acid as solvent. These products showed i.r. absorptions for *O*-acyl groups at ~1750 (C=O) and ~1240 cm^{-1} (C–O) in addition to those of *N*-acyl groups at ~1650 (C=O) and ~1540 cm^{-1} (N–H), and d.s. values of 1.10–1.53 per hexosaminide residue. *O*-Deacetylation of these derivatives was effected by treatment with ethanolic potassium hydroxide. The products were identical with the appropriate derivatives in Table I.

The acylchitosans from the higher fatty acids and benzoic acid exhibit i.r. absorptions for *N*-acyl but not for *O*-acyl groups, and d.s. values of 0.20–1.00 per hexosaminide residue.

EXPERIMENTAL

General. — N.m.r. spectra were recorded at 60 MHz on a Hitachi R-24 spectrometer in DCOOD, using sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)-propionate as internal standard, and i.r. spectra on a Hitachi 215 grating spectrometer (KBr). Specific rotations were recorded on a Yanagimoto OR-50 polarimeter, using a path length of 1 cm.

Elemental analysis was performed at the Elemental Analysis Center of Kyoto University, Kyoto.

Chitin was conventionally isolated[12] from shells of the crab, *Chionecetes opilio* O. Fabricus, and *N*-deacetylation was performed with 40% aqueous sodium hydroxide[4] in the presence of 1% of $NaBH_4$. The resulting chitosan was almost completely *N*-deacetylated (i.r. and n.m.r. spectra) and had $[\alpha]_D^{15}$ −10.5° (*c* 1.3, 10% acetic acid).

N-*Acylation of chitosan.* — (*a*) *In aqueous, methanolic acetic acid.* Chitosan (0.5 g) was dissolved in 10 ml of 10% aqueous acetic acid by shaking at room temperature. The viscous solution was diluted with 40–50 ml of methanol with stirring, and the carboxylic anhydride (2–3 mol. per hexosaminide residue) was added. For the anhydrides of the higher fatty acids (C_{10}–C_{18}), each mixture was heated in a boiling water-bath for a few seconds to afford a clear solution. The solutions, when stored at room temperature overnight, gave rigid gels. Precipitates were produced in the reactions with the anhydrides of some higher fatty acids (C_{14}–C_{18}). A suspension of each product in ~300 ml of methanol was stirred overnight at room temperature. The products were then isolated by freeze-drying of suspensions in distilled water, or by stirring overnight at room temperature with ~300 ml of acetone, then filtering, and washing with ether. The products were dried over P_2O_5 *in vacuo* at 100° for 1–5 h.

(*b*) *In aqueous solutions of fatty acids.* Chitosan (0.1 g) was dissolved in 5 ml of one of the acidic solvents listed in Table II. An excess of carboxylic anhydride (see Table II) was added, and the mixture was left at room temperature overnight. The resulting gels were treated as described above to afford the *N*- and *O*-acylchitosans. The *N*- and *O*-acylchitosans from (*b*) were stirred with 0.5M KOH in ethanol at room temperature overnight. The products were collected, and washed with ethanol and

ether to afford the *N*-acylchitosans (90–94%). The completion of *O*-deacetylation was confirmed by the absence of i.r. absorptions at ~1750 and ~1240 cm^{-1}.

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