Carbohydrate Research, 92 (1981) 323–327
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

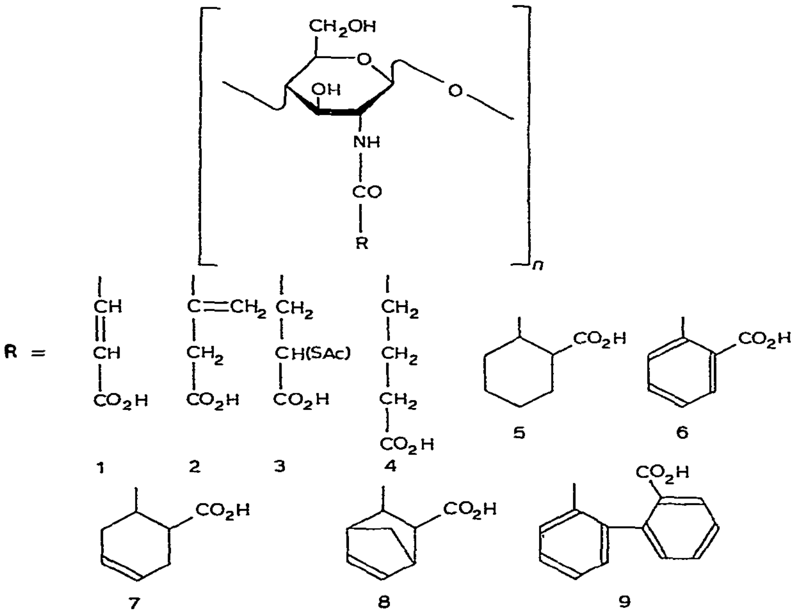
Note

N-(Carboxyacyl)chitosans

SHIGEHIRO HIRANO AND TAMOTSU MORIYASU
Department of Agricultural Biochemistry, Tottori University, Tottori 680 (Japan)
(Received December 1st, 1980; accepted for publication, December 29th, 1980)

2-Acetamido-3-*O*-(1-carboxyethyl)-2-deoxy-β-D-glucosyl residues are present in cell walls of bacteria[1]. *N*-Succinylated derivatives of chitosan [a (1→4)-linked 2-amino-2-deoxy-β-D-glucan] have been reported[2], but little is known[3] about other *N*-(carboxyacyl) derivatives.

The selective *N*-substitution of chitosan with monocarboxylic anhydrides and with aldehydes in aqueous acetic acid–methanol has been reported[4,5]. We now report on some novel *N*-(carboxyacyl) derivatives prepared by the reaction of chitosan with some intramolecular dicarboxylic anhydrides.



Scheme 1. Some *N*-acylchitosans containing carboxylic acids in their *N*-acyl groups. Proposed structures are shown for compounds **2** and **3**.

TABLE I

DATA FOR *N*-ACYLCHITOSANS CONTAINING CARBOXYLIC ACIDS IN THEIR *N*-ACYL GROUPS

Compound[a]	*Gelation*[b]	*Yield (%)*	*D.s.*[c]	$[\alpha]_D^{15}$[f] (c, 0.5, 1% NaOH) (degrees)	*Formula*[d]	*Calc. (%)*			*Found (%)*		
						C	*H*	*N*	*C*	*H*	*N*
1 E	+	74	0.68		$[(X)(C_4H_3O_3)_{0.68}(H)_{0.32}(C_2H_4)_{0.18} \cdot 0.86H_2O]_n$	45.27	6.28	5.46	45.31	6.24	5.46
S			0.68	−24	$[(X)(C_4H_2O_3Na)_{0.68}(H)_{0.32} \cdot 1.96H_2O]_n$	37.66	5.65	5.04	37.58	5.65	5.03
2 E	−	73	0.69		$[(X)(C_5H_5O_3)_{0.69}(H)_{0.31}(C_2H_4)_{0.62} \cdot 0.89H_2O]_n$	47.22	6.68	5.15	47.04	6.65	5.14
S			0.69	−10	$[(X)(C_5H_4O_3Na)_{0.69}(H)_{0.31} \cdot 1.96H_2O]_n$	39.27	5.93	4.85	39.30	5.98	4.71
3 E[e]	+	64	0.56		$[(X)(C_6H_7O_4S)_{0.56}(H)_{0.44}(CH_2)_{0.54} \cdot 1.11H_2O]_n$	41.10	6.14	4.94	40.82	6.49	5.04
S[f]			0.45	−17	$[(X)(C_4H_4O_3SNa)_{0.45}(H)_{0.55} \cdot 1.97H_2O]_n$	37.24	6.53	5.57	37.60	6.25	5.57
4 E	−	67	0.75		$[(X)(C_5H_7O_3)_{0.75}(H)_{0.25}(C_2H_4)_{0.39} \cdot 0.73H_2O]_n$	46.70	6.89	5.17	46.71	6.85	5.17
S			0.75	−12	$[(X)(C_5H_6O_3Na)_{0.75}(H)_{0.25} \cdot 1.68H_2O]_n$	39.90	6.22	4.77	39.90	6.20	4.69
5 E	+	70	0.77		$[(X)(C_8H_{10}O_3)_{0.77}(H)_{0.23}(C_2H_4)_{0.35} \cdot 0.45H_2O]_n$	52.00	6.86	4.72	52.18	6.79	4.72
S			0.77	−8	$[(X)(C_8H_9O_3Na)_{0.77}(H)_{0.23} \cdot 1.53H_2O]_n$	45.14	6.30	4.33	45.00	6.20	4.26
6 E	+	63	0.56		$[(X)(C_8H_5O_3)_{0.56}(H)_{0.44}(C_2H_4)_{0.49} \cdot 0.78H_2O]_n$	50.62	6.21	5.15	50.93	6.29	5.19
S[g]			0.53	−22	$[(X)(C_8H_4O_3Na)_{0.53}(H)_{0.47} \cdot 1.63H_2O]_n$	45.81	5.94	5.22	45.80	5.96	5.16
7 E	+	74	0.80		$[(X)(C_8H_9O_3)_{0.80}(H)_{0.20}(C_2H_4)_{0.80} \cdot 0.64H_2O]_n$	53.09	6.92	4.42	53.14	6.99	4.49
S			0.80	−19	$[(X)(C_8H_8O_3Na)_{0.80}(H)_{0.20} \cdot 1.83H_2O]_n$	44.67	6.12	4.20	44.49	6.12	4.24
8 A[h]	+	70	0.73	−20	$[(X)(C_9H_9O_3)_{0.73}(H)_{0.27} \cdot 0.39H_2O]_n$	52.42	6.17	4.86	52.26	5.99	4.96
9 E	−	56	0.43		$[(X)(C_{14}H_9O_3)_{0.43}(H)_{0.57}(C_2H_4)_{0.43} \cdot 0.75H_2O]_n$	55.09	6.22	4.90	55.14	6.62	5.05
S			0.43	−6	$[(X)(C_{14}H_8O_3Na)_{0.43}(H)_{0.57} \cdot 1.84H_2O]_n$	47.68	6.75	4.63	47.65	6.79	4.63

[a] E, Ethyl or methyl esters; S, sodium salts; A, acids. Compounds **1–5** were partially esterified, and **6**, **7**, and **9** were fully esterified. All of the products were hygroscopic. [b] Key: +, gels formed; −, gels did not form. [c] Determined by C/N ratios of the elemental analyses. [d] X is $C_6H_{10}NO_4$. [e] Isolated as the methyl ester, because methanol was used for its isolation (Calc.: S, 6.33. Found: S, 6.37). The ethyl ester was also prepared in 62% yield {Calc. for $[(C_6H_{10}NO_4)(C_6H_7O_4S)_{0.45}(H)_{0.55}(C_2H_4)_{0.43} \cdot 0.46H_2O]_n$: C, 44.18; H, 6.34; N, 5.39; S, 5.55. Found: C, 44.15; H, 6.34; N, 5.21; S, 5.46}. [f] λ_{max} 308 nm (0.1M NaOH). [g] P.m.r. data (0.1M NaOD in D_2O): δ 7.90 (Ph) and 6.00–3.00 (methyne and methylene protons of the sugar). λ_{max} 309 nm (0.1M NaOH). [h] λ_{max} 300 nm (0.1M NaOH).

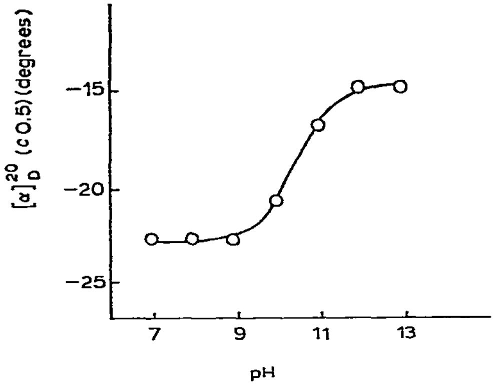


Fig. 1. Effect of pH on the specific rotation of *N*-succinylchitosan (d.s. 0.8/GlcN).

As shown in Scheme 1, nine *N*-acyl derivatives were produced as gels or viscous solutions by the reaction of chitosan variously with excess (3–5 mol/GlcN) of maleic (**1**), itaconic (**2**), (acetylthio)succinic (**3**), glutaric (**4**), cyclohexane-1,2-dicarboxylic (**5**), phthalic (**6**), *cis*-tetrahydrophthalic (**7**), 5-norbornene-2,3-dicarboxylic (**8**), and diphenic (**9**) anhydrides. The products were isolated in 56–76% yields as their esters or acids, and as their sodium salts (Table I). The esters showed i.r. absorptions at $\sim$1710 cm^{-1} and p.m.r. signals for EtO at δ $\sim$1.2 and $\sim$3.7 (0.1M NaOD–D_2O). The products were saponified (**3** was also *S*-deacetylated) by treatment with 0.1M NaOH at room temperature overnight, and the corresponding sodium salts were isolated.

The degree of substitution (d.s.) for *N*-acyl groups was 0.45–0.80 per GlcN, which did not increase even with excess of anhydride (14 mol/GlcN), presumably because of steric hindrance. The unsubstituted amino groups were completely *N*-acetylated with acetic anhydride under the present conditions.

The *N*-acyl derivatives were soluble in 0.1M NaOH. By acidification with 0.1M HCl, white precipitates were produced at pH 4–5, which corresponds to the isoelectric points of the products. The precipitates dissolved or swelled at pH <1. These unique properties reflect the presence of carboxylic and amino groups in the same molecule. The products may exist in extended form in both alkaline and acidic solutions, because of charge repulsions of carboxylic or amino groups, and aggregate at the isoelectric point to form precipitates. Conformational changes may account for the variation of specific rotation of *N*-succinylchitosan (d.s. 0.8/GlcN) with change in pH (Fig. 1).

Compound **3** in 0.1M NaOH solution showed typical absorption for SH groups at λ_{max} 308 nm, on account of their ionisation, and the absorption disappeared in 0.1M HCl solution.

After swelling in pyridine at 50° for 24 h, **6** was treated with acetic anhydride and pyridine (1:1) at 50° for 24 h, and the mixture was boiled for 3 h to give an *O*-acetylated (d.s. 1.4/GlcN) derivative of *N*-acetyl-*N*-phthaloylchitosan (d.s. 0.53:0.47/GlcN); ν_{max}^{KBr} 3600–3400 (OH and NH), 2900 (CH), 1750 and 1230 (C=O and C-O of OAc), 1720 (COOH), 1660 and 1530 (C=O and NH of *N*-acyl), 1120–1000 (C-O), 920 (*β*-D), and 720 cm^{-1} (*o*-Ph). The products were still soluble in 0.1M NaOH, with concomitant *O*-deacetylation. These results suggest that almost no *N*-phthaloyl groups were involved in phthalimido formation[6] and in cross-linking between carboxylic and amino groups, but its cross-links with hydroxyl groups are unknown.

EXPERIMENTAL

Materials and methods. — Chitosan, $[\alpha]_D^{17}$ $-13.6°$ (*c* 0.5, 2% acetic acid), was prepared from chitin (crab shell) by *N*-deacetylation with 40% NaOH in the presence of 0.01% of $NaBH_4$. *N*-Acetyl groups were not detected in the p.m.r. spectrum (D_2O–DCO_2D, 9:1) of the products.

The other analytical methods and materials have been described previously[5].

N-*Acylation of chitosan with intramolecular, dicarboxylic anhydrides.* — A solution of chitosan (0.16 g) in 2% acetic acid (5 ml) was diluted with methanol (30 ml). To the solution was added the corresponding anhydride (3–5 mol/GlcN) dissolved in methanol (10 ml), and the mixture was allowed to stand at room temperature overnight, to afford gels or viscous solutions. The products were suspended in ethanol (150 ml) several times at room temperature, in order to extract excess of reagents. The products were collected by filtration or centrifugation, washed with ethanol and then ether, and dried over P_2O_5 *in vacuo* at 80° for 5 h, to afford precipitates (esters or acids). The products were treated in 0.1M NaOH (25 ml) at room temperature overnight, ethanol (3 vol.) was added, and the precipitates were collected by centrifugation, washed with ethanol, and dried, to afford sodium salts.

D.s. for *N*-acyl groups was determined from the C/N ratio of the elemental analyses, and in part by the p.m.r. spectra (0.1M NaOD in D_2O).

N-*Acetylation of unsubstituted amino groups present in* N-[*(acetylthio)succinyl*]-*chitosan* (3). — A powdered, dry sample of 3 (0.90 g, d.s. 0.45/GlcN; see footnote *e* in Table I) was suspended in ethanol (150 ml), and acetic anhydride (4 ml) was added. The mixture was stirred at room temperature for 8 h. The product was collected by filtration and re-suspended in ethanol (100 ml). The suspension was filtered, washed with ethanol and then ether, and dried over P_2O_5 *in vacuo* at 100° for 3 h, to afford *N*-acetyl-*N*-[(acetylthio)succinyl]chitosan as the ethyl ester (0.93 g); ν_{max}^{KBr} 3500–3200 (OH and NH), 1720 (ester), 1650 and 1550 (C=O and NH of *N*-acyl), 1090–1000 (C-O), and 890 cm^{-1} (*β*-D).

Anal. Calc. for $[(C_6H_{10}NO_4)(C_6H_7O_4S)_{0.45}(C_2H_3O)_{0.55}(C_2H_4)_{0.45} \cdot 1.25\ H_2O]_n$: C, 43.15; H, 6.46; N, 4.70; S, 4.85. Found: C, 43.15; H, 6.56; N, 4.89; S, 4.76.

ACKNOWLEDGMENTS

This work was supported by research grants from the Nissan Science Foundation and from the Ministry of Education, Japan.

REFERENCES

1 J. L. Strominger and J. M. Ghuysen, *Science*, 156 (1967) 213–221.
2 R. Yamaguchi, Y. Arai, T. Itoh, and S. Hirano, *Carbohydr. Res.*, 88 (1981) 172–175.
3 G. Vanlerberghe and H. Sebag, Ger. Pat., 2,222,733 (1972); *Chem. Abstr.*, 78 (1973) 477,664.
4 S. Hirano, Y. Ohe, and H. Ono, *Carbohydr. Res.*, 47 (1976) 315–320.
5 S. Hirano, N. Matsuda, O. Miura, and H. Iwaki, *Carbohydr. Res.*, 71 (1979) 339–343.
6 S. Hirano, *Carbohydr. Res.*, 16 (1971) 229–231.