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Note

Preparation and chelating properties of derivatives of chitosan and 1,3-dicarbonyl compounds

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We have recently reported [1] the preparation of a derivative of chitosan (**1**) and 2,4-pentanedione, which showed a strong chelating capacity with Cu(II) and Co(II). The new polymer proved to have the expected structure of an *N*-acylvinyl derivative of chitosan with a degree of substitution (ds) of 1.00. The possibility of practical application of this derivative prompted us to prepare other derivatives of chitosan and 1,3-dicarbonyl compounds, and to study their chelating properties. Besides simple 1,3-dicarbonyl compounds, such as 1-phenyl-1,3-butanedione (**2**), benzoylacetaldehyde (**3**), and methyl acetoacetate (**4**), ethoxymethylene derivatives [diethyl ethoxymethylenemalonate (**5**), 3-ethoxymethylene-2,4-pentanedione (**6**)] and 2-anilinomethylene-5,5-dimethyl-1,3-cyclohexanedione (**7**) have also been treated with chitosan. Previous work [2–6] had shown that the reaction of **2**–**7** with 2-amino-2-deoxy-D-glucose, the monomer of chitosan, affords *N*-acylvinyl derivatives of the amino sugar.

As in the reaction with 2,4-pentanedione, chitosan (**1**; percentage of acetylated amino groups, expressed [7] as NAc content, 13%) was treated with 3 mol of **2**–**7** per 2-amino-2-deoxyglucosyl residue to give colourless (except for **9**, which was yellow), water-insoluble, amorphous polysaccharide amino-enone derivatives (**8**–**13**) in high yields (83–96%). The elemental analysis data and the IR spectra (two bands at 1605–1677 and 1541–1596 cm^{-1} for the amino-enone system) agreed with the structures **8**–**13** (NAc 13%, ds 1.00). CPMAS spectra of **8**, **9**, **11**, and **13** (Table 1), as compared with those of the monomers **14**–**15** [2] as solids, and glycosides **16** [4] and **17** in solution, confirmed the structure assignment, the main differences being the shifts of the anomeric carbon (β linkages for the chitosan derivatives and **17**, but α for **14**–**16**) and of C-4 (due to the *O*-substitution in the polymer).

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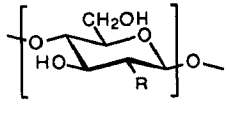
Table 1
^{13}C NMR data (δ, ppm) for the polymers **8**, **9**, **11**, and **13**, and the monomers **14** and **15** as solids (at 75.4 MHz), and for **16** and **17** as solutions in Me_2SO-d_6 (at 50.3 MHz)

Assignment	Compound							
	(Solid)						(In solution)	
	8	**9**	**11**	**13**	**14**	**15**	**16**	**17**
$2\times CH_3CH_2O$			13.9					
$CH_3C{=}C$	21.0				18.3		20.0	20.1
					19.8			
					20.8			
$(CH_3)_2C$				28.4				
$(CH_3)_2C$				31.1				
2″ $CH_2C{=}O$				50.4				
CH_3O							54.8	56.6
2″CH_3CH_2O			60.2 [a]					
C-2	61.0 [b]	61.8	60.2 [a]	*	57.6	57.1	57.5	59.9
C-6	61.0 [b]	65.3	60.2 [a]	*	59.4	58.5	60.9	61.0
C-4	84.5	83.3	90.0 [c]	*	70.1	72.4 [d]	70.5	70.6
C-3	75.5	75.3 [e]	75.1 [f]	*	72.2	72.4 [d]	73.1	75.5
C-5	80.0	75.3 [e]	75.1 [f]	*	74.0	72.4 [d]	73.4	76.8
					75.5			
C-2′	94.0	92.1	90.0 [c]	102.1	96.3	92.6 [g]	98.3	91.9
				100.8				
C-1 (α)					91.8	92.6 [g]	91.9	
C-1 (β)	105.0	103.8	103.7	107.4				102.7
Ph	127.2	127.7			127.8	128.6	126.8	126.7
	139.1	138.6			137.2	130.9	128.4	128.4
					139.9	139.4	130.6	130.6
					141.9	140.3	140.3	140.1
C-1′	167.9	156.5	169.1 [h]	*	166.4	158.8	165.4	166.4
					169.2			
C=O (unchelated)			169.1 [h]	193.2				
C=O (3′)	187.4	190.8	169.1 [h]	197.4	183.8	192.7	185.8	185.8
					184.6			
					189.6			

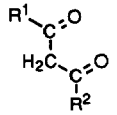
[a–h] Overlapped signals.
* Signals indistinguishable from baseline noise.

Total hydrolysis of **8–13** required heating in M HCl for 0.5–4 h and yielded 85–92% of chitosan hydrochloride, while the 2,4-pentanedione derivative was hydrolysed [1] by M HCl even at room temperature. The released 1,3-dicarbonyl compound was determined only for **8**, which gave, after treatment to reflux with M HCl for 1 h, 90% of **2**. Compounds **8–13** proved to be stable at room temperature in the pH range 0–10, except **10**, which was stable only between pH 3 and 9.

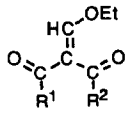
The introduction of the enone group on the nitrogen of each 2-amino-2-deoxy-glucosyl residue of chitosan, which had resulted in a high enhancement of the chelating capacity [1] of chitosan after its reaction with 2,4-pentanedione, seems to have little or no effect when the reagents are **2–7**. Table 2 records the relative amounts of Cu(II) and Co(II) chelated by **8–13** in their reactions with Cu(II)



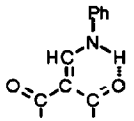
1 R = NH_2 (idealised)



2 R^1 = Me, R^2 = Ph
3 R^1 = H, R^2 = Ph
4 R^1 = Me, R^2 = OMe

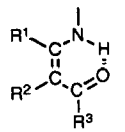


5 R^1 = R^2 = OEt
6 R^1 = R^2 = Me

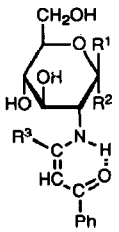


7

8-13 R =



8 R^1 = Me, R^2 = H, R^3 = Ph
9 R^1 = R^2 = H, R^3 = Ph
10 R^1 = Me, R^2 = H, R^3 = OMe
11 R^1 = H, R^2 = EtOCO, R^3 = OEt
12 R^1 = H, R^2 = MeCO, R^3 = Me
13 R^1 = H, R^2, R^3 = -CO-CH_2-$C(Me)_2$-CH_2-



14 R^1 = H, R^2 = OH, R^3 = Me
15 R^1 = H, R^2 = OH, R^3 = H
16 R^1 = H, R^2 = OMe, R^3 = Me
17 R^1 = OMe, R^2 = H, R^3 = Me

acetate and Co(II) acetate, respectively, in aqueous solution at pH 5. Only **13** is able to complex 60% [for Cu(II)] or 43% [for Co(II)] of the metal required for 1:2 chelates (metal–2-amino-2-deoxyglucosyl residue) (cf. 94% for the 2,4-pentanedione derivative [1]). Compound **12** shows medium values of Cu(II)- and Co(II)-chelating capacities (39 and 37%, respectively), while the capacities shown by **8–11** are poor [18–30% for Cu(II) and 11.5–23% for Co(II)]. A possible explanation of the decrease of the chelating capacity in comparison with that of the 2,4-pentanedione derivative may be the steric effect of bulky groups such as the phenyl group in **8** and **9**, or the decrease in the π-electron density in the delocalised amino-enone system by cross-conjugated groups as in **10–13**, more evident for esters (**10** and **11**) than for ketones (**12** and **13**). The stabilities of the complexes metal–**8–13** have been studied by treating them with chelating agents of known stability constants. The results are similar to those obtained for the complexes of Cu(II) and Co(II) with the 2,4-pentanedione derivative of chitosan; thus, all the stability constants lie in the range 10^{12}–10^{14} for the Cu(II) complexes and 10^{5}–10^{9} for the Co(II) complexes.

In conclusion, the new chitosan derivatives **12** and **13** possess an acceptable chelating capacity against Cu(II) and Co(II), but less than the 2,4-pentanedione derivative, while **8–11** chelate only minor amounts of both ions.

1. Experimental

General methods.—Solvents were evaporated in vacuo at < 40°C. Freeze-drying was performed with a Virtis Freeze-mobile-3 apparatus. Chitosan from krill was

supplied by the Sea Fisheries Institute (Gdynia, Poland) and its NAc content (13%) was determined by elemental analysis and IR spectroscopy [8]. IR spectra were recorded for films or KBr discs with a Bomem MB-120 spectrophotometer. ^{13}C CPMAS NMR spectra (75.4 MHz) were recorded with a Bruker MSL-300 spectrometer and ^{13}C NMR spectra (50.3 MHz) on solutions with a Varian XL-200 spectrometer. The Cu and Co contents of metal complexes were determined by atomic absorption analysis with a Perkin–Elmer 2380 apparatus.

Reaction of chitosan (1) with 1,3-dicarbonyl compounds. Preparation of N-*acylvinyl derivatives of chitosan.*—A solution of the 1,3-dicarbonyl compound (**2**–**4**), or the ethoxymethylene (**5** or **6**), or the anilinomethylene (**7**) derivatives (16.5 mmol) in MeOH (40 mL; 100 mL for **7**) was added to a stirred solution of **1** (1.0 g, 5.5 mmol) in 1:1 MeOH–aq 10% AcOH (80 mL). The mixture was kept at room temperature until gelification took place (0.5–48 h). The soft gel was washed succesively with MeOH, EtOH, and ether, then dialysed against water for 24 h, freeze-dried, and dried at 100°C/1 torr. Analytical samples were dried at 100°C/0.02 torr.

The following products were obtained in this manner.

(1 → 4)-2-[*Z*-(2-Benzoyl-1-methylvinyl)amino]-2-deoxy-β-D-glucan (**8**; 1.63 g, 96%) [from 1-phenyl-1,3-butanedione (**2**, 2.67 g), after 2 h]; ν_{max} 3443 (NH and OH), 1605 and 1541 cm^{-1} (intramolecularly bonded O=C–C=C–N–H). For the ^{13}C CPMAS NMR data, see Table 1. Anal. Calcd for $[(C_{16}H_{19}NO_5)_{0.87}\ (C_8H_{13}NO_5)_{0.13} \cdot H_2O]_n$: C, 57.95; H, 6.57; N, 4.52. Found: C, 58.20; H, 6.28; N, 4.48.

(1 → 4)-2-[*Z*-(2-Benzoylvinyl)amino]-2-deoxy-β-D-glucan (**9**; 1.52 g, 96%) [from benzoylacetaldehyde (**3**, 2.44 g), after 0.5 h]; ν_{max} 3420 (NH and OH), 1630 and 1584 cm^{-1} (O=C–C=C–N–H). For the ^{13}C CPMAS NMR data, see Table 1. Anal. Calcd for $[(C_{15}H_{17}NO_5)_{0.87}\ (C_8H_{13}NO_5)_{0.13} \cdot 0.6H_2O]_n$: C, 58.22; H, 6.13; N, 4.82. Found: C, 58.49; H, 6.28; N, 4.41.

(1 → 4)-2-Deoxy-2-[*Z*-(1-methyl-2-methoxycarbonylvinyl)amino]-β-D-glucan (**10**; 1.24 g, 85%) [from methyl acetoacetate (**4**, 1.91 g), after 48 h]; ν_{max} 3410 (NH and OH), 1644 and 1572 cm^{-1} (O=C–C=C–N–H). Anal. Calcd for $[(C_{11}H_{17}NO_6)_{0.87}\ (C_8H_{13}NO_5)_{0.13} \cdot 0.7\ H_2O]_n$: C, 48.26; H, 6.81; N, 5.26. Found: C, 48.16; H, 6.32; N, 5.33.

(1 → 4)-2-Deoxy-2-[2,2-diethoxycarbonylvinyl)amino]-β-D-glucan (**11**; 1.24 g, 91%) [from diethyl ethoxymethylenemalonate (**5**, 3.57 g), after 12 h]; ν_{max} 3458 (NH and OH), 1710sh (free ester C=O), 1653br and 1580sh cm^{-1} (intramolecularly bonded O=C–C=C–N–H). For the ^{13}C CPMAS NMR data, see Table 1. Anal. Calcd for $[(C_{14}H_{21}NO_8)_{0.87}\ (C_8H_{13}NO_5)_{0.13} \cdot 0.5H_2O]_n$: C, 49.06; H, 6.53; N, 4.33. Found: C, 48.92; H, 6.67; N, 3.86.

(1 → 4)-2-[(2,2-Diacetylvinyl)amino]-2-deoxy-β-D-glucan (**12**; 1.33 g, 83%) [from 3-ethoxymethylene-2,4-pentanedione (**6**, 2.58 g), after 48 h]; ν_{max} 3395 (NH and OH) and 1611br cm^{-1} (free C=O and intramolecularly bonded O=C–C=C–N–H). Anal. Calcd for $[(C_{12}H_{17}NO_6)_{0.87}\ (C_8H_{13}NO_5)_{0.13} \cdot 1.5H_2O]_n$: C, 47.64; H, 6.78; N, 4.84. Found: C, 48.07; H, 6.72; N, 5.19.

(1 → 4)-2-Deoxy-[(4,4-dimethyl-2,6-dioxocyclohexylidenemethyl)amino]-β-D-glucan (**13**; 1.47 g, 85%) [from 2-anilinomethylene-5,5-dimethyl-1,3-cyclohexane-

dione (**7**, 4.01 g), after 6 h]; ν_{max} 3425 (NH and OH), 1677 (free C=O), and 1596 cm^{-1} (intramolecularly bonded O=C–C=C–N–H). For the ^{13}C CPMAS NMR data, see Table 1. Anal. Calcd for $[(C_{15}H_{21}NO_6)_{0.87}\ (C_8H_{13}NO_5)_{0.13} \cdot H_2O]_n$: C, 53.67; H, 7.02; N, 4.44. Found: C, 54.10; H, 7.32; N, 4.72.

Hydrolysis of the N-*acylvinyl derivatives of chitosan* (**8–13**).—A solution of the compound **8–13** (0.100 g) in M HCl (20 mL) was heated to reflux for the time stated below, then dialysed against water, freeze-dried, and dried in vacuo to give chitosan hydrochloride, identified by comparison of its IR spectrum with that of an authentic sample. The following amounts of chitosan hydrochloride were obtained. From **8** (reflux time, 1 h): 0.063 g (90% yield). From **9** (reflux time, 4 h): 0.068 g (92%). From **10** (reflux time, 0.5 h): 0.074 g (90%). From **11** (reflux time, 4 h): 0.058 g (88%). From **12** (reflux time, 4 h): 0.065 g (87%). From **13** (reflux time, 4 h): 0.059 g (85%).

A gravimetric determination of the released 1,3-dicarbonyl compound was made for **8**. A solution of **8** (0.200 g) in M HCl (40 mL) was heated to reflux for 1 h, then extracted with ether (3 × 40 mL). The combined ethereal extracts were dried ($MgSO_4$) and evaporated to give 1-phenyl-1,3-butanedione (**2**; 0.085 g, 90%).

Stability of the N-*acylvinyl derivatives of chitosan* (**8–13**).—Suspensions of **8–13** (0.02 g) in buffer solutions of pH 0–10 (10 mL) were shaken at room temperature. Aliquots (1.0 mL) of the supernatant solution (its total volume was maintained each time by addition of 1.0 mL of buffer) were taken at intervals (0–48 h) and diluted to 25.0 mL, and the remaining solid was collected and studied by IR spectroscopy. The recovered solid was thus identified as the starting material, except for **10** in buffers of pH 0, 1, 2, and 10, for which the IR spectrum of the recovered solid was similar to that of chitosan or chitosan salts. The corresponding content of β-dicarbonyl compound was investigated by measuring the UV absorption of the filtrate. No β-dicarbonyl compound was found in the pH range 3–9 for **10**, or in the total pH range studied for the rest (**8**, **9**, and **11–13**).

Chelate formation of **8–13** *with Cu(II) and Co(II)*.—A suspension of the appropriate chitosan derivative (**8–13**, 0.100 g) in satd aq Cu(II) acetate or Co(II) acetate (50 mL) was stirred vigorously at room temperature for 12 h. The solid was collected, washed thoroughly with water, dialysed against water, freeze-dried, and dried in vacuo.

Each complex (0.100 g) was digested with hot concd HNO_3. The residue was dissolved in water, the solution was diluted to 100 mL, and aliquots (1.0 mL) were used for atomic absorption analysis. The experimental ratios metal–polysaccharide are recorded in Table 2. Calculated for a 1:2 metal–2-amino-2-deoxyglucosyl unit stoichiometry: 0.435 mol/mol.

*Stability of the complexes Cu(II)–***8–13** *and Co(II)–***8–13**.—A suspension of each complex (10.0 mg) in water (5 mL) was stirred at room temperature for 48 h with an aqueous or methanolic solution (5 mL) of a stoichiometric amount of each ligand (L) (in brackets, the stability constants, k_s) [9].

Disodium EDTA [17.2 mg for the formation of the CuL_2 complex (k_s $10^{18.8}$), or 16.8 mg for the formation of the CoL_2 complex (k_s $10^{16.3}$)] gave the corresponding free polymer (**8–13**).

Table 2
Relative amounts of metal chelated by the new polymers **8–13**

Chelate	Cu(II)		Co(II)	
	mg/g of chitosan	mol/mol of monomer	mg/g of chitosan	mol/mol of monomer
Metal–**8**	32.5	0.09	18	0.05
Metal–**9**	28.6	0.08	15	0.05
Metal–**10**	47	0.13	32	0.10
Metal–**11**	42	0.12	28	0.08
Metal–**12**	60	0.17	51	0.16
Metal–**13**	90	0.26	66	0.21

With 2,4-pentanedione [4.6 mg for the CuL_2 complex (k_s $10^{14.3}$), or 4.5 mg for the CoL_2 complex (k_s $10^{8.9}$)], all the Cu(II)–**8–13** complexes afforded the violet solid Cu(II) acetylacetonate immediately, and all the Co(II)–**8–13** complexes gave the free polymers **8–13**, respectively.

Ammonia had no effect when bubbled through suspensions of the Cu(II)–**8–13** complexes in MeOH. Likewise, oxalic acid dihydrate [5.9 mg for the CuL_2 complex (k_s $10^{8.9}$), or 5.7 mg for the CoL_2 complex (k_s $10^{5.8}$)] and NaOAc trihydrate [9.3 mg for the CuL_3 complex (k_s $10^{3.1}$), or 6.0 mg for the CoL_2 complex (k_s $10^{1.5}$)] did not react with any of the Cu(II)– or Co(II)–**8–13** complexes.

Methyl 2-[Z-(2-benzoyl-1-methylvinyl)amino]-2-deoxy-β-D-glucopyranoside (**17**). —To a solution of methyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (0.27 g, 1.2 mmol) in MeOH (6 mL) were added 1-phenyl-1,3-butanedione (0.60 g, 3.7 mmol) and Et_3N (0.4 mL). The mixture was boiled under reflux, with monitoring of the reaction by TLC on Alugram Sil G/UV_{254} (Macherey–Nagel) (10:1 CH_2Cl_2–MeOH). After 6 h, the solvent was evaporated to dryness. Column chromatography of the residue on Silica Gel 60 (Merck, 63–200 μm) (10:1 CH_2Cl_2–MeOH) afforded pure **17** (0.20 g, 50%), isolated as a syrup; $[\alpha]_D^{20}$ +24° (*c* 1, MeOH); $\lambda_{max}^{H_2O}$ 343 nm (ε 20 300); ν_{max}^{KBr} 3429 (OH), 1583br and 1543 cm^{-1} (amino-enone system). NMR data [$(CD_3)_2SO$]: ^1H, δ 7.84–7.45 (m, 5 H, phenyl), 5.77 (s, 1 H, H-2′), 5.35 (d, 1 H, HO), 5.17 (d, 1 H, HO), 4.63 (t, 1 H, HO-6), 4.35 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 3.8–3.0 (m, 6 H, H-2, H-3, H-4, H-5, 2 H-6), 3.39 (s, 3 H, MeO), and 2.08 (s, 3 H, Me-C); ^{13}C: see Table 1. Anal. Calcd for $C_{17}H_{23}NO_6$: C, 60.52; H, 6.87; N, 4.15. Found: C, 60.27; H, 6.75; N, 4.27.

Acknowledgments

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