Carbohydrate Research, 233 (1992) 255–259
Elsevier Science Publishers B.V., Amsterdam

Note

A derivative of chitosan and 2,4-pentanedione with strong chelating properties

Manuel Gómez-Guillén, Antonio Gómez-Sánchez and María-Eloísa Martín-Zamora

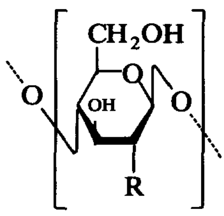
Departamento de Química Orgánica "Profesor García González" and Instituto de la Grasa y sus Derivados (C.S.I.C.), Universidad de Sevilla, Apartado de Correos No. 553, 41071 Seville (Spain)

(Received October 22nd, 1991; accepted March 16th, 1992)

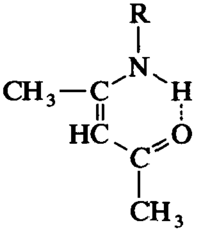
The chelating properties of chitosan (**1**) can be enhanced when the amino groups are substituted to give derivatives (e.g., $NHCH_2CO_2H$) that have a stronger chelating ability[1,2]. Some of these derivatives have industrial[2] and medical[3] applications. Several *N*-benzylidenechitosans have been described[4], among which the *N*-salicylidene derivative[5] (**2**) had increased chelating capacity against Cu(II). Since derivatives (**3**) from amines and 1,3-dicarbonyl compounds are good metal chelators[6], the preparation and chelating properties of similar derivatives of chitosan were investigated. 2-Amino-2-deoxy-D-glucose reacts with 1,3-dicarbonyl compounds to afford *N*-acylvinyl derivatives, for example, **4** from 2,4-pentanedione[7]. We now report on the corresponding derivative (**5**) of chitosan (**1**).

Treatment of chitosan (**1**, NAc 13%) with 3 mol of 2,4 pentanedione per 2-amino-2-deoxyglucosyl residue yielded (94%) a white, water-insoluble, amorphous polysaccharide amino-enone derivative **5**. The elemental analysis agreed with structure **5** (NAc 13%) and a degree of substitution (ds) of 1.00. The IR spectrum contained bands at 1607 and 1566 cm^{-1} for the intramolecularly bonded amino-enone system, as shown[7] by the monomeric analogue **4**. The structure **5** was confirmed by comparison of its solid-state CP-MAS spectrum with that of **4** and with that of the methyl β-pyranoside derivative **6** in solution (Table I). Although treatment of **5** with M HCl at room temperature regenerated chitosan (90–92%) and 2,4-pentanedione (94%) in agreement with a ds of 1.00, **5** was stable at room temperature in the pH range 3–9.

Correspondence to: Professor A. Gómez-Sánchez, Departamento de Química Orgánica "Profesor García González" and Instituto de la Grasa y sus Derivados (C.S.I.S.), Universidad de Sevilla, Apartado de Correos No. 553, 41071 Seville, Spain.

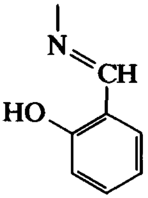


1 R = NH_2 (idealised)

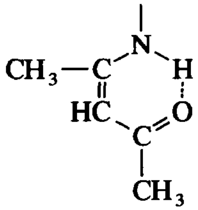


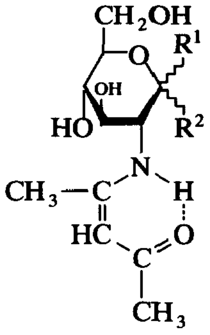
3

2 R =



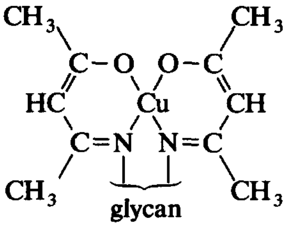
5 R =





4 R^1, R^2 = H, OH

6 R^1 = OMe (β), R^2 = H



7

Copper(II) acetate and Co(II) acetate reacted readily with **5** in aqueous solution at pH 5 to produce the coloured complexes, Cu(II)-**5** and Co(II)-**5**, which incorporated 94% of the metal required for chelates with metal : 2-amino-2-deoxyglucosyl residue ratios of 1 : 2, corresponding to the partial structure **7** as found[6] for metal chelates derived from the simple ligands **3**. The copper-chelating capacity of **5** [2.28 mmol of Cu(II) per g] is enhanced by a factor of 38 over that [0.06 mmol of Cu(II) per g] recorded[5] for chitosan and is similar to those [0.62 and 3.29 mmol of Cu(II) per g] reported[5] for **2** under different conditions. The metals could not be displaced from Cu(II)-**5** and Co(II)-**5** by ammonia, sodium acetate, or oxalic acid, but were totally or partially displaced by EDTA and 2,4-pentanedione. These results indicate a stability constant (k_s) in the ranges 10^{12}–10^{14} for Cu(II)-**5** and 10^5–10^9 for Co(II)-**5**.

Because of its enhanced chelating ability and its easy and inexpensive preparation, **5** may find application in those areas where **1** and some of its derivatives have proved to be useful.

EXPERIMENTAL

General methods.—Chitosan (**1**) from krill was supplied by the Sea Fisheries Institute (Gdynia, Poland) and its NAc content (13%) was determined by elemental analysis and IR spectroscopy[9].

TABLE I

^{13}C NMR data (δ, ppm) for **4** and **5** as solids (at 75.4 MHz) and for **6** as a solution in Me_2SO-d_6 (at 50.3 MHz)

Assignment	Compound		
	(Solid)		(In solution)
	4	**5**	**6**
CH_3C=C	20.13	19.97	19.58
CH_3C=O (4′)	27.97	27.82	28.81
CH_3O	–	–	56.56
C-2	57.43	60.88	59.59
C-6	60.49	60.88	60.97
C-4	69.31	84.61 [a]	70.60
C-3	70.43	75.51	75.58
C-5	75.98	79.50	76.78
C-1α	91.31	–	–
C-2′	95.29	98.75	95.42
C-1β	96.31	105.53	102.82
C-1′	166.43	168.00	164.01
C=O (3′)	193.23	195.35	193.32

[a] The chemical shift reflects the (1 → 4) linkages; cf. 82.9 and 81.1 ppm for chitin and chitosan[8], respectively.

Anal. Calcd for $[(C_6H_{11}NO_4)_{0.87}(C_8H_{13}NO_5)_{0.13} \cdot 0.8H_2O]_n$: C, 41.53; H, 7.16; N, 7.74. Found: C, 41.21; H, 6.77; N, 7.68.

IR spectra were recorded for KBr discs with a Bomem MB-120 spectrophotometer and compounds were identified by comparison of IR spectra. ^{13}C CP-MAS 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.

2,4-Pentanedione was determined by spectrophotometry of its Fe(III) chelate[10] with a Beckman DU-7 apparatus; Cu and Co contents of the metal complexes were determined by atomic absorption analysis with a Perkin–Elmer 2380 apparatus.

(1 → 4)-2-[Z-(2-Acetyl-1-methylvinyl)amino]-2-deoxy-β-D-glucan (**5**).—(*a*) *Preparation.* To a stirred solution of **1** (1.0 g, 5.5 mmol) in 1:1 MeOH–aq 10% acetic acid (80 mL) was added a solution of 2,4-pentanedione (1.65 g, 16.5 mmol) in MeOH. After 4 h, the soft yellow gel was washed successively with MeOH, EtOH and ether, then dialysed against water for 24 h, freeze-dried, and dried at 100°/1 torr to give **5** (1.32 g, 94%) as a white powder. Analytical samples, dried at 100°/0.02 torr, had ν_{max} 3441 (NH and OH), 1607 and 1566 cm^{-1} (intramolecularly bonded O=C–C=C–N–H). For the ^{13}C CP-MAS NMR data, see Table I.

Anal. Calcd for $[(C_{11}H_{17}NO_5)_{0.87}(C_8H_{13}NO_5)_{0.13} \cdot H_2O]_n$: C, 49.76; H, 7.27; N, 5.47. Found: C, 49.71; H, 7.16; N, 5.06.

(*b*) *Hydrolysis.* A solution of **5** (0.10 g) in M HCl (20 mL) was stored at 20° for 1 h, then dialysed against water, freeze-dried, and dried in vacuo to give chitosan hydrochloride (0.078 g, 92%); 94% of 2,4-pentanedione was present in the hydrolysate.

(*c*) *Stability*. Suspensions of **5** (0.02 g) in buffer solutions of pH 0–10 were shaken at room temperature. Aliquots (1.0 mL) were taken at intervals (0–48 h) and diluted to 25.0 mL, and the 2,4-pentanedione content was determined. No β-diketone was released in the pH range 3–9.

(*d*) *Chelate formation with Cu(II) and Co(II)*. A suspension of **5** (0.10 g) in satd aq Cu(II) acetate or Co(II) acetate (50 mL) was stirred vigorously at room temperature for 12 h. The solid, Cu(II)-**5** or Co(II)-**5**, was collected, washed thoroughly with water, dialysed against water, freeze-dried, and dried in vacuo.

Each complex (0.10 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 ratios metal : polysaccharide were as follows: Cu(II)-**5**, 145 mg of Cu(II)/g of chitosan [0.41 mol Cu(II)/mol 2-amino-2-deoxyglucosyl unit]; and Co(II)-**5**, 133 mg of Co(II)/g of chitosan [0.41 mol Co(II)/mol 2-amino-2-deoxyglucosyl unit]. Calculated for a 1 : 2 metal : 2-amino-2-deoxyglucosyl unit stoichiometry: 0.435 mol/mol.

*Stability of Cu(II)-***5** *and Co(II)-***5**.—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)[11].

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 **5**.

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 $10^{8.9}$)], Cu(II)-**5** afforded the violet solid Cu(II) acetylacetonate immediately, and Co(II)-**5** gave **5**.

Ammonia had no effect when bubbled through a suspension of Cu(II)-**5** 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 sodium acetate 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 Cu(II)-**5** or Co(II)-**5**.

ACKNOWLEDGMENTS

We thank the Comisión Interministerial de Ciencia y Tecnología (C.I.C.Y.T.) for financial support (Project PB87-0454), Dr. M.M. Brzeski of the Sea Fisheries Institute (Gdynia, Poland) for a generous gift of chitosan, the Instituto de Ciencias de Materiales (C.S.I.C., Madrid) for the ^{13}C CP-MAS NMR spectra, the Departamento de Química Analítica (Universidad de Sevilla) for the microanalyses and atomic absorption analyses, and the Ministerio de Educación y Ciencia of Spain for a fellowship (to M.E.M.Z.).

REFERENCES

1 R.A.A. Muzzarelli, F. Tanfani, M. Emanuelli, and S. Mariotti, *Carbohydr. Res.*, 107 (1982) 199–214; R.A.A. Muzzarelli, F. Tanfani, and M. Emanuelli, *Biotechnol. Bioeng.*, 27 (1985) 1115–1121.

2 R.A.A. Muzzarelli, in R. Muzzarelli, C. Jeuniaux, and G.W. Gooday (Eds.), *Chitin in Nature and Technology*, Plenum Press, New York, 1986, pp 321–330.

3 G. Biagini, A. Pugnaloni, G. Frongia, G. Gazzanelli, C. Lough, and R.A.A. Muzzarelli, in G. Skjäk-Braek, T. Anthonsen, and P. Sanford (Eds.), *Chitin and Chitosan*, Elsevier Applied Science, London, 1989, pp 671–677.

4 L.A. Nud'ga, E.A. Plisko, and S.N. Danilov, *Zh. Obshch. Khim.*, 43 (1973) 2752–2756; S. Hirano, N. Matsuda, O. Miura, and H. Iwaki, *Carbohydr. Res.*, 71 (1979) 339–343.

5 L.D. Hall and M. Yalpani, *Carbohydr. Res.*, 83 (1980) c5–c7; L.D. Hall and T.K. Lim, *ibid.*, 148 (1986) 13–23.

6 P.J. McCarthy, R.J. Hovey, K. Ueno, and A.E. Martell, *J. Am. Chem. Soc.*, 77 (1955) 5820–5824; R.J. Hovey, J.J. O'Connell, and A.E. Martell, *ibid.*, 81 (1959) 3189–3192.

7 F. García González, A. Gómez Sánchez, and M.I. Goñi de Rey, *Carbohydr. Res.*, 1 (1965) 261–273.

8 M. Takai, Y. Shimizu, J. Hayashi, Y. Uraki, and S. Tokura, ref. 3, pp 431–436.

9 M. Miya, R. Iwamoto, S. Yoshikawa, and S. Mima, *Int. J. Biol. Macromol.*, 2 (1980) 323–324.

10 I.M. Korenman and M.I. Gryaznova, *Tr. Khim. Khim. Tekhnol.*, (1970) 72–73; *Chem. Abstr.*, 76 (1972) 135583.

11 A. Ringbom, *Formación de Complejos en Química Analítica*, Alhambra, Madrid, 1979, pp 351–379.