Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Research 341 (2006) 351–354
Carbohydrate RESEARCH

Novel derivatives of chitosan and their antifungal activities in vitro

Zhanyong Guo,[a,b] Rong Chen,[c] Ronge Xing,[a,b] Song Liu,[a,b] Huahua Yu,[a,b] Pibo Wang,[a,b] Cuiping Li[a,b] and Pengcheng Li[a,*]

[a]*Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China*
[b]*Graduate School of the Chinese Academy of Sciences, Beijing 100039, China*
[c]*College of Chemistry and Chemical Engineering, Ocean University of China, Qingdao 266003, China*

Received 11 July 2005; accepted 1 November 2005
Available online 15 December 2005

Abstract—Three kinds of Schiff bases of carboxymethyl chitosan (CMCTS) were prepared, and their antifungal activities were assessed according to Jasso de Rodríguez's method. The results indicated that 2-(2-hydroxybenzylideneamino)-6-carboxymethylchitosan (HNCMCTS) and 2-(5-chloro-2-hydroxybenzylideneamino)-6-carboxymethylchitosan (HCCMCTS) had better inhibitory effects than those of chitosan or CMCTS against *Fusarium oxysporium* f. sp. *vasinfectum*, *Alternaria solani*, and *Valsa mali*.

Keywords: Schiff bases; Antifungal activity; Carboxymethyl chitosan

1. Introduction

As one of the most abundant naturally occurring compounds, chitosan has attracted people's attention on account of its unique physiochemical characteristics and its bioactivities.[1–4] However, because of its poor solubility in water, the use of chitosan is limited in many fields. In order to improve its aqueous solubility, many derivatives of chitosan have been synthesized, and carboxymethyl chitosan (CMCTS) is the most important one. Generally, CMCTS is synthesized by the reaction of chitosan and chloroacetic acid in 2-propanol in the presence of KOH. CMCTS can easily dissolve in water, which enlarges the scope of the use of chitosan.[5,6] However, there seems to be little further study on the modification of CMCTS. In the CMCTS molecule, the NH_2 groups have a degree of substitution (DS) of 0.1–0.2,[7] so there are also enough NH_2 groups to take part in other reactions for CMCTS that is obtained from highly deacetylated chitosan.

Antifungal activity is one of the most important bioactivities of chitosan, and earlier studies have reported that chitosan can reduce the growth of phytopathogenic fungi, which are harmful to field crops, fruit, and vegetables.[8–14] In studies on the antifungal activity of chitosan, researchers have focused most of their attention on the molecular weight and the degree of deacetylation of chitosan, which affect its activities. But, little work has been reported on the antifungal activities of the derivatives of chitosan.

In this paper, three kinds of Schiff bases of CMCTS are reported, as well as their antifungal activities against *Valsa mali* (*V. mali*), *Alternaria solani* (*A. solani*), and *Fusarium oxysporium* f. sp. *vasinfectum* (*F. oxysporium* f. sp. *vasinfectum*) from a study that employed the method of Jasso de Rodríguez and co-workers.[15]

2. Materials and methods

2.1. Materials

Chitosan was purchased from Qingdao Baicheng Biochemical Corp. (China). Its degree of deacetylation

Abbreviation: HNCMCTS, 2-(2-hydroxy-5-nitrobenzylideneamino)-6-carboxymethylchitosan; HCCMCTS, 2-(5-chloro-2-hydroxybenzylideneamino)-6-carboxymethylchitosan; HCMCTS, 2-(2-hydroxybenzylidenamino)-6-carboxymethylchitosan.

* Corresponding author. Tel.: +86 532 82898707; fax: +86 532 82968951; e-mail addresses: guozhanyong@ms.qdio.ac.cn; pcli@ms.qdio.ac.cn

0008-6215/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2005.11.002

was 97%, and the viscosity average-molecular weight was 2.0×10^5. Salicylaldehyde was purchased from Fluka Chemical Co. The IR spectrum was measured by a Nicolet Magne-Avatar 360 instrument using KBr disks. The elemental analyses (C, H, N) were performed on a Carlo–Erba 1106 elemental analyzer. The other reagents were of analytical grade and used without further purification. Three crop-threatening pathogenic fungi *V. mali*, *A. solani*, and *F. oxysporium* f. sp. *vasinfectum* were provided by Professor Xiangli Dong (Laiyang Agricultural College).

2.2. The synthesis of the Schiff bases of CMCTS

CMCTS was prepared according to a previously reported method,[7] and the syntheses of 5-chloro-2-hydroxybenzaldehyde and 2-hydroxy-5-nitrobenzaldehyde were, respectively, carried out according to the procedures of Liu and Zhao,[16] and Zhang.[17] The Schiff bases of CMCTS were synthesized as shown in Scheme 1. CMCTS (3 g) was dissolved in H_2O (100 mL) and various aldehydes were added with stirring at 25 °C. After 1 h, the solution was precipitated in acetone and the precipitate was filtered. The unreacted aldehydes and other inorganic products were extracted in a Soxhlet apparatus with EtOH and ether for 2 days. The Schiff bases were obtained by lyophilization of their aqueous solutions.

2.3. Antifungal assays

Antifungal assays were performed based on the method of Jasso de Rodríguez and co-workers.[15] Different concentrations of chitosan, CMCTS, HNCMCTS, HCCMCTS, and HCMCTS (5, 50, and 500 ppm) were, respectively, added to sterilized potato dextrose agar (PDA). The test plates were incubated at 27 °C after transferring the mycelium of fungi. When the mycelium of fungi reached the edges of the control plate (without added the samples), the antifungal index was calculated as follows:

$$\text{Antifungal index}(\%) = (1 - D_a/D_b) \times 100,$$

where D_a is the diameter of the growth zone in the test plates and D_b is the diameter of growth zone in the control plate. Each experiment was performed three times, and the data were averaged. The Scheffe method was used to evaluate the differences in antifungal index in antifungal tests. Results with $P < 0.05$ were considered statistically significant.[18]

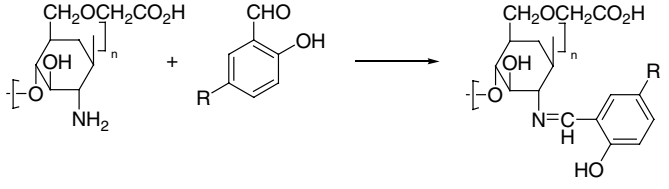
3. Results and discussion

3.1. Structure and physicochemical characteristics of the derivatives of chitosan

The results of the elemental analyses and the color of the Schiff bases are listed in Table 1, and the IR spectra of chitosan, CMCTS, and the Schiff bases are shown in Figure 1. As shown in Figure 1, the IR spectra of chitosan shows peaks assigned to the saccharide structure at 897.85 and 1154.98 cm^{-1} and characteristic amino peak at 1600.03 cm^{-1}. CMCTS is confirmed by the absorption bands at 1600.57 [$\nu_{sym}(CO_2)$] and 1416.06 cm^{-1} [$\nu_{as}(CO_2)$].[19] The elemental analyses results indicate that the N,O-substitution degree of CMCTS is about 0.96, and the degree of N-substitution is about 0.17 as measured by the method of Chen and co-workers.[7] Compared to the IR spectra of chitosan and CMCTS, the Schiff bases have strong peaks at 1630–1660 cm^{-1}, which are assigned to the characteristic absorbance of the imino groups [C=N].[20] Moreover, there are strong peaks at about 1570, 1500, 1450, and 750 cm^{-1} corresponding to the phenyl groups.[21] These results indicate that the Schiff bases of CMCTS are obtained, and the substitution degree of HNCMCTS, HCCMCTS, and HCMCTS is calculated as 0.51, 0.59, and 0.60, respectively, per glucosamine unit from the results of the elemental analyses.

Table 1. The elemental analysis results and the color of chitosan, CMCTS, and the Schiff bases of CMCTS

Compounds	Elemental analyses (%)			Color
	C	N	H	
Chitosan	44.72	8.70	6.83	White
CMCTS	43.86	6.46	5.96	White
HNCMCTS	47.11	7.22	4.94	Yellow
HCCMCTS	48.49	4.80	4.99	Yellow
HCMCTS	52.22	5.00	5.48	Yellow



HNCMCTS: R = NO_2; HCCMCTS: R = Cl; HCMCTS: R = H

Scheme 1. Synthetic pathway for the Schiff bases of CMCTS.

3.2. Antifungal activity

3.2.1. Antifungal activities of chitosan, CMCTS, and the Schiff bases of CMCTS against *F. oxysporium* f. sp. *vasinfectum*. *F. oxysporum* f. sp. *vasinfectum* can cause Cotton Fusarium Wilt, which is highly destructive and economically limiting to the production of quality cotton. It has been proven that chitosan has an inhibitory effect against *F. oxysporium* f. sp. *vasinfectum*,[22] which is also confirmed in our tests (inhibitory index = 14.3% at 500 ppm) as shown in Figure 2. CMCTS has also a less potent antifungal activity against *F. oxysporium* f. sp. *vasinfectum* with an inhibitory index of 9.1% at 500 ppm. HNCMCTS and HCCMCTS have better antifungal activities than that of chitosan and CMCMTS, and the inhibitory index is 31.2% and 43.0%, respectively, at 500 ppm. However, the antifungal activity of HCMCTS is not as potent as that of HNCMCTS and HCCMCTS. Above-mentioned results indicate that the increased antifungal activities of HNCMCTS and HCCMCTS against *F. oxysporium* f. sp. *vasinfectum* may be due to 2-hydroxy-5-nitrobenzylideneamino and 5-chloro-2-hydroxybenzylideneamino groups grafted onto the chitosan chain. This is further supported by the antifungal activities of HNCMCTS and HCCMCTS at 50 ppm.

3.2.2. Antifungal activities of chitosan, CMCTS, and the Schiff bases of CMCTS against *A. solani*. *A. solani* causes early blight of potato, tomato, capsicum, and brinjal, and the outputs of these vegetables are greatly affected by these diseases. As shown in Figure 3, chitosan, CMCTS, and all the Schiff bases have antifungal activities against *A. solani*, and the antifungal activities increase with increasing concentration. For HNCMCTS and HCCMCTS, at a concentration of 50 ppm, the antifungal index was 2.9% and 5.6%, respectively. It should be for the same reason that the increased antifungal activities are due to 2-hydroxy-5-nitrobenzylideneamino and 5-chloro-2-hydroxybenzylideneamino groups. As antifungal groups, the nitro and chloro groups are used in many fungicides such as pentachloronitrobenzene (PCNB) and chlorothalonil.[23] But these fungicides have pronounced toxicities and their residues in the environment have been developing as serious problems. When these groups are grafted onto chitosan, they should be released slowly and may meet the requirements of environmental safety.

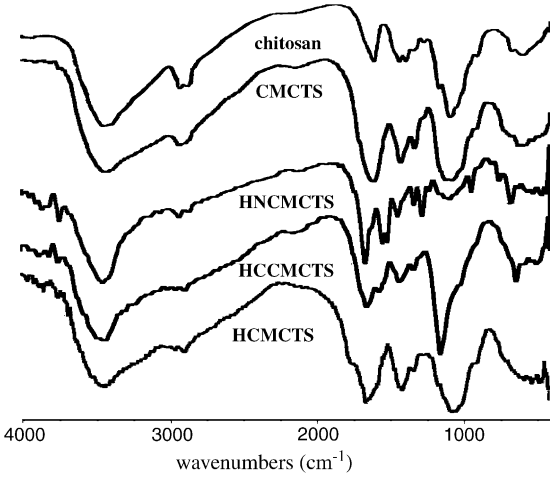


Figure 1. IR spectral data for chitosan, CMCTS, and the Schiff bases of CMCTS.

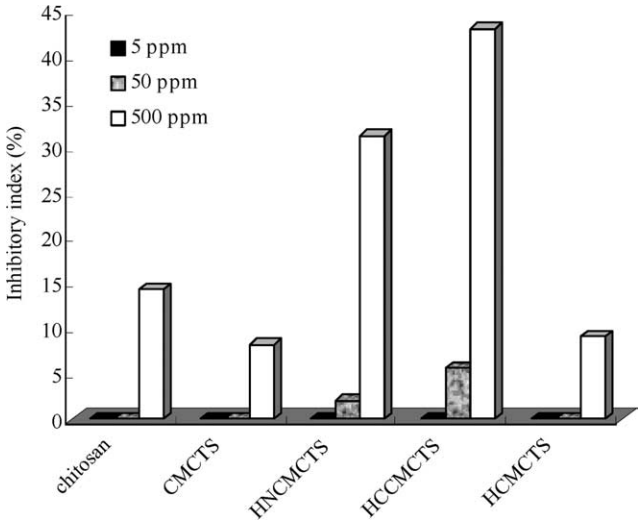


Figure 2. The antifungal activities of chitosan, CMCTS, and the Schiff bases of CMCTS against *Fusarium oxysporium* f. sp. *vasinfectum*.

3.2.3. Antifungal activities of chitosan, CMCTS, and the Schiff bases of CMCTS against *V. mali*. As shown in Figure 4, chitosan and its derivatives have antifungal

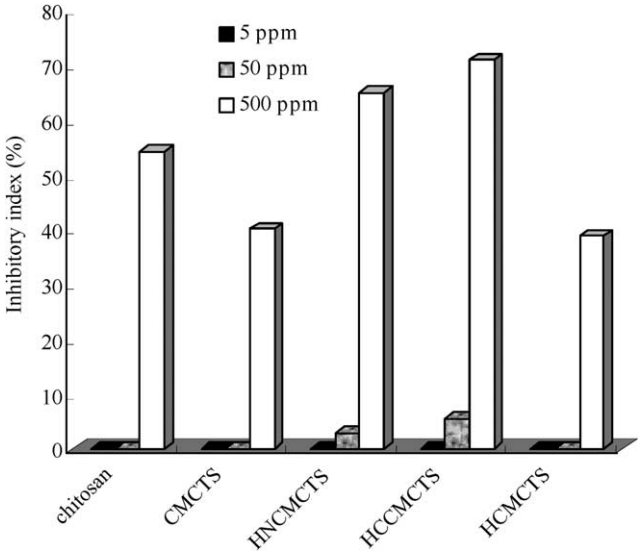


Figure 3. The antifungal activities of chitosan, CMCTS, and the Schiff bases of CMCTS against *Alternaria solani*.

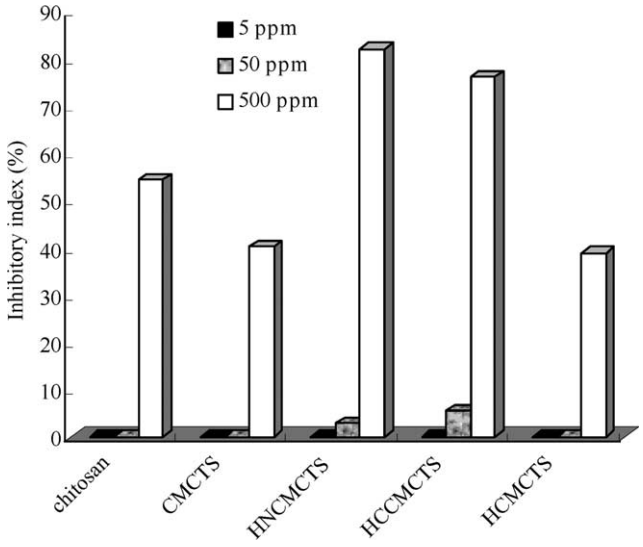


Figure 4. The antifungal activities of chitosan, CMCTS, and the Schiff bases of CMCTS against *Valsa mali*.

activities at 500 ppm against *V. mali*, but there are no inhibitory effects at 5 ppm. At concentrations of 50 ppm, the inhibitory indices for HNCMCTS and HCCMCTS are 2.9% and 5.6%, respectively, in contrast to chitosan, CMCTS, and HCMCTS, all of which have no antifungal activities at 50 ppm. The increased antifungal activities of HNCMCTS and HCCMCTS may be also due to 2-hydroxy-5-nitrobenzylideneamino and 5-chloro-2-hydroxylbenzylideneamino groups.

4. Conclusions

Antifungal activity is one of the most important bioactivities of chitosan and it will be improved in some of the derivatives, which is determined by the groups grafted to chitosan. CMCTS dissolves in water easily and water solubility remains after appropriate modification. Some of the derivatives of CMCTS have good bioactivities, and from our experiments, two kinds of Schiff bases of CMCTS have better antifungal activities than those of chitosan and CMCTS against *V. mali*, *A. solani* and *F. oxysporium* f. sp. *vasinfectum*. Further studies on these compounds are warranted.

Acknowledgements

This work was financially supported by the Innovational Foundation of Chinese Academy of Science (KZCX3-SW-215). The authors are grateful to Professor Xiangli Dong of the Laiyang Agricultural College, for their technical assistance.

References

1. Allan, C. R.; Hadwiger, L. A. *Exp. Mycol.* **1979**, *3*, 285–287.
2. Hadwiger, L. A.; Loschke, D. C. *Exp. Mycol.* **1981**, *71*, 756–762.
3. Stossel, P.; Leuba, J. L. *Phytopathol. Z.* **1984**, *111*, 82–90.
4. EI Ghaouth, A.; Arul, J.; Asselin, A. *Phytopathology* **1992**, *82*, 398–402.
5. Muzzarelli, R. A. A. *Carbohydr. Polym.* **1988**, *8*, 1–22.
6. Muzzarelli, R. A. A.; Tanfani, F.; Emanuelli, M.; Mariotti, S. *Carbohydr. Res.* **1982**, *107*, 199–214.
7. Chen, L. Y.; Du, Y. M.; Liu, Y. *J. Wuhan Univ. (Nat. Sci. Ed.)* **2000**, *16*, 191–194.
8. Roller, S.; Covill, N. *Int. J. Food Microbiol.* **1999**, *47*, 67–77.
9. Ben-Shalom, N.; Ardi, R.; Pinto, R.; Aki, C.; Fallik, E. *Crop Protect.* **2003**, *22*, 285–290.
10. Benhamou, N. *Trends Plant Sci.* **1996**, *1*, 233–240.
11. Benhamou, N. *Phytopathology* **1992**, *82*, 1185–1193.
12. Li, J. Y.; Li, H. Y. *J. Chin. Electron. Microsc. Soc.* **2002**, *21*, 138–140.
13. Yu, H. S.; Chen, Y. X. *Phytophylacica Sinica* **2002**, *29*, 295–299.
14. Sudarshan, N. R.; Hoover, D. G.; Knorr, D. G. *Food Biotechnol.* **1992**, *6*, 257–272.
15. Jasso de Rodríguez, D.; Hernández-Castillo, D.; Rodríguez-García, R.; Angulo-Sánchez, J. L. *Ind. Crop. Prod.* **2005**, *21*, 81–87.
16. Liu, C. Y.; Zhao, Q. Q. *Chin. J. Pharm.* **2001**, *32*, 37.
17. Zhang, S. H. *Guangxi Sci.* **2003**, *10*, 284–285.
18. Ramos, A. C. S.; Dantas Neto, A. A.; Castro Dantas, T. N. C. *Braz. J. Chem. Eng.* **1997**, *14*, 159–165.
19. Sun, T.; Xie, W. M.; Xu, P. X. *Carbohydr. Polym.* **2004**, *58*, 379–382.
20. Ajay, K. S.; Chebrolu, P. R.; Pauli, K. S.; Erkki, K.; Kari, R. *Carbohydr. Res.* **2001**, *335*, 33–43.
21. Matsumoto, M.; Shimizu, T.; Kondo, K. *Sep. Purif. Technol.* **2002**, *29*, 229–233.
22. Zhang, M.; Tan, T. W.; Yuan, H. Z.; Rui, C. H. *J. Beijing Univ. Chem. Tech.* **2003**, *30*, 13–16.
23. Sha, J. J. *Handbook of Chemical Product*, 3rd ed.; Chemical Industry: Beijing, 1994, pp 190–192.