

perature. It crystallized readily from 2-propanol and melted at 268–269°.

*Anal.* Calcd. for  $C_{23}H_{42}O_6$ : C, 70.85; H, 8.92; for  $C_{30}H_{46}O_6$ : C, 71.68; H, 9.22. Found: C, 70.55, 70.80, 70.80; H, 8.48, 8.47, 8.70.

Terminal methyl: 13.5%, equivalent to 5.0 methyl groups capable of yielding acetic acid, assuming 85% recovery. Zerevitinov determination: 0.774, 0.814, 0.807% H, equiv-

alent to 3.67, 3.86, and 3.83 reactive hydrogen atoms per molecule.

Ultraviolet absorption spectrum:  $\lambda_{max}$ , 270  $m\mu$ ,  $\log \epsilon$  3.92; end absorption at 210  $m\mu$ ,  $\log \epsilon$  3.48; minimum at 232  $m\mu$ ,  $\log \epsilon$  3.04. Infrared spectrum: maxima of strong intensity at 5.85 and 6.17  $\mu$ , and one of very high intensity at 5.93  $\mu$ .

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

## Chitosan Nitrate<sup>1</sup>

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Received August 1, 1958

The nitrate ester-salt, the nitrate and perchlorate salts, and the nitrate ester derivatives of chitosan (essentially *N*-deacetylated chitin) have been prepared in stable form. The perchlorate salt of chitosan nitrate was prepared but was quite unstable.

Chitin, a condensation polymer of 2-acetamido-2-deoxy-D-glucopyranose believed to be linked  $\beta$ -(1→4), when subjected to the action of strong alkali<sup>2</sup> affords the corresponding *N*-deacetylated polymer, chitosan. Most chitosan preparations still contain considerable amounts of the *N*-acetyl function. The configurationally *trans* orientation of the acetamido and hydroxyl groups on carbons two and three of the polymeric units makes the acetyl function extremely difficult to remove with alkali. However, considerable success has recently been achieved<sup>3</sup> in reducing the acetyl content of chitosan to a negligible value. Graded hydrolysis of the completely deacetylated chitosan has been employed for the isolation of the D-glucosamine oligosaccharides<sup>3,4</sup> which confirm the  $\beta$ -(1→4) character of the original chitin.

In our investigation of the polymeric nitrate derivatives of chitosan, we chose to employ a reprecipitated preparation of about 85% *N*-deacetylation rather than to subject the polymer to the degradative action required to raise this value significantly. Part of this work was, however, carried out with a chitosan of 97% *N*-deacetylation.

Suspensions of these chitosans in glacial acetic acid containing either perchloric or nitric acid afforded excellent yields of the corresponding water-soluble amine salts. In some cases, sufficient acetic anhydride was added to combine with the water introduced by the mineral acids.

Chitosan (85% free amine) was found to dissolve

in absolute nitric acid but the resulting chitosan nitrate was nearly identical to that obtained employing absolute nitric acid admixed with acetic acid and acetic anhydride which reacts heterogeneously with chitosan. Both reaction media afforded the nitric acid salt of chitosan nitrate ester in which approximately 85% of the two available hydroxyl functions were esterified, corresponding to a degree of substitution of 1.7. The heterogeneous method resulted in somewhat higher yields and avoided difficulties in the isolation of the product from the reaction mixture. The conversion of the nitric acid salt of chitosan nitrate ester to the free amino nitrate (unchanged ester content) was accomplished in 80% yield by careful treatment of the salt, dissolved in 50% aqueous acetone, with dilute alkali.

The free amino chitosan nitrate ester was treated with an anhydrous solution of perchloric acid in acetic acid to form the perchlorate salt of chitosan nitrate ester, all samples of which were unstable at room temperature, decomposing slowly in most cases and in one instance with detonation.

Similar results were obtained when chitosan containing 97% free amine groups was employed. However, the drastic conditions utilized to obtain this chitosan were reflected by the deviations in the analyses obtained for the derivatives and by the decreased stability of the perchlorate salt of this chitosan nitrate ester.

At least two factors appear to contribute to the observed instability of the derivatives of chitosan nitrate ester. The polymeric derivatives are structurally related to the nitrate ester-salt derivatives of the mono-, di- and tri-ethanolamines, which monomeric crystalline substances decrease in stability in the order given.<sup>5</sup> The alkaline saponi-

(1) Carried out under contracts DA-33-019-ORD-163 and -727 between The Ohio State University Research Foundation (Projects 458 and 496) and the United States Army Ordnance Corps under the supervision of the Ballistic Research Laboratories, Aberdeen Proving Ground, Md.

(2) G. W. Rigby, U. S. Patent 2,040,879 (1936).

(3) Sylvia T. Horowitz, S. Roseman, and H. J. Blumenthal, *J. Am. Chem. Soc.*, **79**, 5046 (1957).

(4) S. A. Barker, A. B. Foster, M. Stacey, and J. M. Webber, *J. Chem. Soc.*, 2218 (1958).

(5) J. Barbrière, *Bull. soc. chim. France*, [5], **11**, 470 (1944); Aubry, *Mem. poudres*, **25**, 189 (1932); *Chem. Abstr.*, **27**, 4083 (1933).

fication of chitin to chitosan may introduce small amounts of certain functions, carbonyl in particular, which are believed to have adverse effects on the stability of polymeric nitrate esters.

## EXPERIMENTAL

*Chitosan.* Crude chitosan<sup>6</sup> (38 g.), prepared from shrimp chitin by the method of Rigby,<sup>2</sup> was dissolved in 1 liter of 10% aqueous acetic acid. The mixture was centrifuged after 24 hr. and the clear supernatant was treated dropwise with 40% aqueous sodium hydroxide. The white flocculent precipitate obtained at pH 7 was recovered by centrifugation and washed repeatedly with water, ethanol, and ether; yield 28.5 g.

*Anal.* Calcd. for  $C_6H_9O_4(NHCOCH_3)_{0.15}(NH_2)_{0.85}$ : C, 45.18; H, 6.80; N, 8.37;  $CH_3CO$ , 3.86. Found: C, 44.95; H, 6.97; N, 7.58;  $CH_3CO$ ,<sup>7</sup> 4.16.

Attempts to obtain fractions of this material with reduced acetyl content by partial solution, using aqueous acetic acid, were not successful.

An amount of 2.0 g. of this chitosan was stirred under nitrogen with 20 g. of 40% sodium hydroxide at 90° for 1 hr. The resulting material was recovered by centrifugation and washed as above; yield 1.8 g.

*Anal.* Calcd. for  $C_6H_9O_4(NH_2)$ : C, 44.72; H, 6.88; N, 8.69;  $CH_3CO$ , 0.0. Found: C, 44.38; H, 7.02; N, 7.59;  $CH_3CO$ ,<sup>7</sup> 0.8.

*Chitosan salts.* Chitosan (2.0 g., 85% free amine) was suspended in a mixture of 132 ml. of glacial acetic acid and 33 ml. of 60% perchloric acid. The suspension was stirred at 8° for 16 hr. prior to filtration, washing with ether, and drying to afford chitosan perchlorate; yield 3.2 g. (99%).

*Anal.* Calcd. for  $C_6H_9O_4(NHCOCH_3)_{0.15}(NH_3^+ClO_4^-)_{0.85}$ : Cl, 11.90;  $HClO_4$ , 33.8. Found: Cl, 11.92;  $HClO_4$ , 32.1 (titration).<sup>8</sup>

Substitution of 12 ml. of 70% nitric acid for the perchloric acid produced the nitrate salt; yield 2.5 g. (94%).

*Anal.* Calcd. for  $C_6H_9O_4(NHCOCH_3)_{0.15}(NH_3^+NO_3^-)_{0.85}$ : N, 11.73; N (nitrate), 5.39;  $HNO_3$ , 24.2. Found: N, 11.58 (Dumas); N, 5.51 (nitrometer);<sup>9</sup>  $HNO_3$ ,<sup>8</sup> 24.4.

Similarly, chitosan (97% free amine), treated with acetic acid and perchloric acid, yielded the corresponding perchlorate salt.

*Anal.* Calcd. for  $C_6H_9O_4(NH_3^+ClO_4^-)$ :  $HClO_4$ , 37.4. Found:  $HClO_4$ , 37.1.<sup>8</sup>

(6) Kindly furnished by Prof. J. F. Haskins of this department.

(7) M. L. Wolfrom, D. I. Weisblat, J. V. Karabinos, W. H. McNeely, and J. McLean, *J. Am. Chem. Soc.*, **65**, 2084 (1945); see also A. Chaney and M. L. Wolfrom, *Anal. Chem.*, **28**, 1614 (1956).

(8) Analyses for mineral acid content were carried out in water or aqueous acetone by titration with alkali to the phenolphthalein endpoint.

(9) W. W. Scott, "Standard Methods of Chemical Analysis," N. H. Furman, ed., D. Van Nostrand Co., Inc., New York, N. Y., 1939, pp. 650-655.

The chitosan salts were soluble in water and ethanol. They decomposed explosively on heating over a direct flame but were stable during storage at room temperature.

*Nitration of chitosan.* Chitosan (200 mg., 85% free amine) was stirred into a mixture of 10 ml. of acetic anhydride, 10 ml. of acetic acid, and 13 ml. of absolute nitric acid at 0-5°. The reaction was terminated after 5.5 hr. at 0-5° by centrifugation. The residue was washed with acetic acid and ether; yield 330 mg. (90%) of the nitric acid salt of chitosan nitrate ester.

*Anal.* Calcd. for  $C_6H_7O_2(ONO_2)_{1.65}(OH)_{0.35}(NHCOCH_3)_{0.15}(NH_3^+NO_3^-)_{0.85}$ : N (nitrate), 11.9;  $HNO_3$ , 18.1. Found: N,<sup>9</sup> 11.2;  $HNO_3$ , see below.

Another sample, 2.0 g., was added to 100 ml. of absolute nitric acid. The mixture was stirred at 0-5° for 1 hr. before the homogeneous solution was poured slowly into 1000 ml. of cool acetic acid with added cooling. The flocculent precipitate was recovered by centrifugation and washed as before; yield 3.1 g. (85%),  $[\alpha]^{25}_D +43^\circ$  (c 1, acetone-water 1:1 by vol.).

*Anal.* Found: N,<sup>9</sup> 11.9;  $HNO_3$ ,<sup>8</sup> 18.6.

The above nitrate salt-ester (1.7 g.) was dissolved in 50% (by vol.) aqueous acetone. The solution was neutralized with 0.3 N alkali to pH 7 (indicator paper). Centrifugation and washing with water, ethanol, and ether afforded the free amino chitosan nitrate ester; yield 1.1 g. (80%).

*Anal.* Calcd. for  $C_6H_7O_2(ONO_2)_{1.65}(OH)_{0.35}(NHCOCH_3)_{0.15}(NH_2)_{0.85}$ : N (nitrate), 9.6. Found: N,<sup>9</sup> 9.8;  $HNO_3$ ,<sup>8</sup> trace.

This product was insoluble in acetone, water or in mixtures of these solvents.

A sample of 97% free amine chitosan was nitrated with absolute nitric acid as described; yield 76%.

*Anal.* Calcd. for  $C_6H_7O_2(ONO_2)_{1.75}(OH)_{0.25}(NH_3^+NO_3^-)$ : N (nitrate), 12.7;  $HNO_3$ , 20.8. Found: N,<sup>9</sup> 12.6;  $HNO_3$ ,<sup>8</sup> 18.8.

This nitrate salt was treated with alkali as before to produce the free amine nitrate ester; yield 93%.

*Anal.* Calcd. for  $C_6H_7O_2(ONO_2)_{1.75}(OH)_{0.25}(NH_2)$ : N (nitrate), 10.2. Found: N,<sup>9</sup> 10.0.

*Salts of chitosan nitrate.* Chitosan nitrate ester (940 mg., 85% free amine) was added to a 3% excess of perchloric acid in acetic acid; the water introduced by the perchloric acid was previously removed by the addition of the calculated amount of acetic anhydride. The resulting gel was stirred for 90 min. at room temperature. The solid was recovered by centrifugation, dissolved in abs. ethanol, and the solution poured into ether. The precipitate of perchlorate salt was centrifuged, washed with ether, and dried; yield 870 mg. (55%).

*Anal.* Calcd. for  $C_6H_7O_2(ONO_2)_{1.65}(OH)_{0.35}(NHCOCH_3)_{0.15}(NH_3^+ClO_4^-)_{0.85}$ : Cl, 9.2; N (nitrate), 7.1;  $HClO_4$ , 26.1. Found: Cl, 9.0; N,<sup>9</sup> 7.0;  $HClO_4$ ,<sup>8</sup> 30.8.

This sample gave evidence of advanced decomposition after 14 weeks storage at room temperature. Other similar preparations ignited or detonated spontaneously during storage (1 to 4 weeks). Samples of this perchlorate prepared from 97% free amine chitosan were not sufficiently stable to permit analysis.

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