

Thiolation of Starch and Other Polysaccharides*

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Synopsis

Thiolated starches with a degree of substitution of 0.01-0.6 were obtained by pyrolysis of starch dithiobis(thioformates) followed by saponification. The pyrolytic decomposition of dithiobis(thioformates) followed two pathways: (1) to thionocarbonate

$$\begin{array}{c} \text{S} \\ || \\ \text{ROCOR} \end{array}$$
, carbon disulfide, and sulfur, and (2) to dithiocarbonate (ROCSR), carbonyl sulfide, and sulfur. Only the second pathway affords thiolation. Thiol groups were determined by Ellman's reagent, by sulfur analyses, and, following acetylation, by the ratio of O-acetyl versus S-acetyl absorption in nuclear magnetic resonance spectra. The polysaccharides cellulose, dextran, and xylan were thiolated by the same procedure.

INTRODUCTION

A goal of the Northern Laboratory is to increase the industrial consumption of starch through new or expanded markets. As one approach, we modified starch by introducing "reactive sites" for crosslinking and for covalently bonding other polymers. Starches or modified starches containing xanthate,^{1,2} carbonate,^{3,4} and epoxypropyl⁵ reactive sites have been described by Laboratory scientists. Although starches possessing thiol groups have been of considerable interest, no simple method has been available for replacing hydroxyl groups of starch with thiol groups.

Previously, amylose had been thiolated at the 6-position by displacement of tosyloxy groups with thiocyanate followed by reduction with lithium aluminum hydride⁶; and cellulose, at the 6-position by displacement of tosyloxy groups with sodium thiosulfate or potassium thiolacetate.⁷ We now have developed a comparatively facile route that converts starch-OH to starch-SH. We also have successfully applied this new procedure to cellulose, dextran, and xylan. Repeating units of these polysaccharides are sketched in Figure 1. Main steps of the new procedure include oxidation of

* Presented at 164th National Meeting of American Chemical Society, New York, New York, August 27-September 1, 1972.

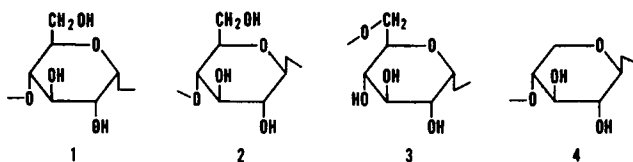
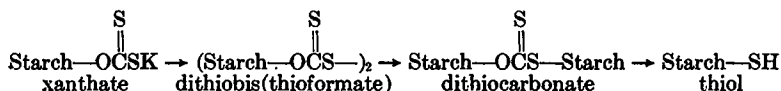


Fig. 1. Main repeating units of the polysaccharides: (1) starch, (2) cellulose, (3) dextran, and (4) xylan.

polysaccharide xanthate to dithiobis(thioformate) followed by pyrolysis and alkali treatment:



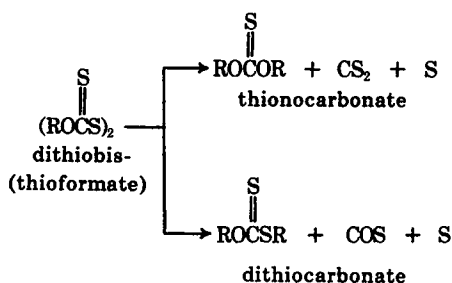
Previously, dithiobis(thioformates) of 1,2:3,4-di-O-isopropylidene- α -D-galactopyranose and 1,2:5,6-di-O-isopropylidene- α -D-glucofuranose were shown to decompose thermally with or without pyridine⁸ to give the



corresponding dimeric thionocarbonate (ROCOR). It was also shown that vicinal diol groups can be easily converted to cyclic thionocarbonates via rearrangement of the corresponding dithiobis(thioformates) with evolution of carbon disulfide.³ Certain dithiobis(thioformates) decomposed by a different pathway. Bis(methyl-2,3,4-tri-O-methyl- α -D-glucopyranoside) 6,6'-dithiobis(thioformate) and methyl-2,3-di-O-methyl- α -D-glucopyranoside 4,6-[dithiobis(thioformate)] in pyridine gave the corresponding 6,6'-



dithiocarbonate (ROCSR) and the cyclic 4,6-dithiocarbonate, respectively, along with carbonyl sulfide. Evolution of carbon disulfide indicated a rearrangement to thionocarbonate and evolution of carbonyl sulfide indicated a rearrangement to dithiocarbonate. Saponification of thionocarbonate led to the parent alcohols, whereas saponification of dithiocarbonate led to alcohol and thiol:



Starch dithiobis(thioformates), prepared by sodium nitrite oxidation of xanthates of degree of substitution (D.S.) 0.1-3, were pyrolyzed. Gases,

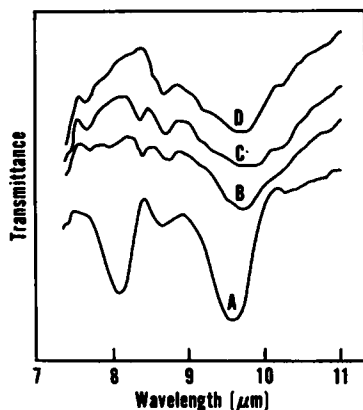


Fig. 2. Infrared spectra of starch dithiobis (thioformate) (A, 43.0% S) and its pyrolysis product (Nujol mulls): (B) A after heating at 220°C for 20 min; (C) B after treatment with *p*-chlorothiophenol; (D) C after treatment with sodium hydroxide and neutralization.

analyzed as the piperidine complexes,⁹ were mixtures of carbon disulfide and carbonyl sulfide in a varying ratio according to conditions.

That formation of dithiocarbonate did occur was further evidenced by infrared (IR) and ultraviolet (UV) spectra. The dithiobis(thioformate) group exhibits broad absorption bands⁹ near 8.1 and 9.6 μm as shown for starch dithiobis(thioformate) prepared from a xanthate of D.S. 3 (Fig. 2, spectrum A). Pyrolysis of this compound at 200°C for 90 min gave gases that indicated 77% decomposition and the ratio of carbonyl sulfide:carbon disulfide of 1:1. The IR spectrum of the pyrolyzate (spectrum B) revealed a weak absorption of 8.1 μm for residual dithiobis(thioformate) and a new band at 8.4 μm characteristic for dithiocarbonate.⁹ The 8.4 μm band remained after treatment of the pyrolyzate with *p*-chlorothiophenol to decompose residual dithiobis(thioformate)¹⁰ (spectrum C), but disappeared upon subsequent treatment with base to form the thiol (spectrum D). When a portion of the product after *p*-chlorothiophenol treatment was digested with α -amylase, the UV spectrum showed absorption maximum at 283 nm for dithiocarbonate.¹¹ Saponification of the pyrolyzed product followed by acetolysis gave a product with absorptions in the IR spectrum at 5.7 μm for O-acetyl and at 5.9 μm for S-acetyl. The sulfur content of the acetate was near theory for that expected from the amount of carbonyl sulfide collected during pyrolysis. Moreover, the nuclear magnetic resonance (NMR) spectrum of the acetate revealed the presence of O-acetyl (τ 8.0) and S-acetyl (τ 7.7) absorptions in a ratio which agreed well with that expected from the sulfur content. When the saponification-acetylation procedures were repeated with unpyrolyzed starch dithiobis(thioformate), the product showed only O-acetyl by IR and contained no sulfur. Treatment of the pyrolyzed-saponified product with Ellman's reagent¹² [5,5'-dithiobis(2-nitrobenzoic acid)] revealed the presence of thiol. This

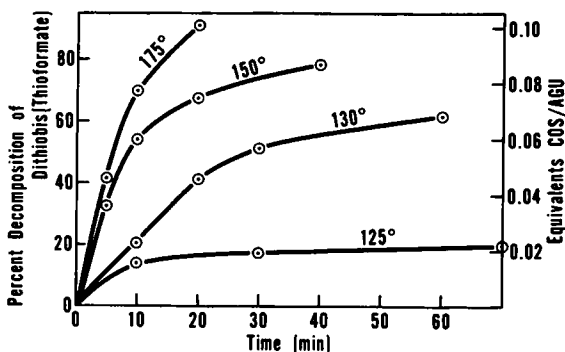


Fig. 3. Influence of temperature upon decomposition of starch dithiobis(thioformate) derived from xanthate degree of substitution (D.S.) 0.95.

procedure is very sensitive and can detect thiol groups in starch with a D.S. as low as 0.01.

The decomposition of dithiobis(thioformates) was studied over a wide range of reaction conditions. Effects of temperature upon decomposition of a dithiobis(thioformate) derived from xanthate D.S. 0.95 are plotted in Figure 3. Decomposition is nearly complete after 30 min at 175°C, but only a partial decomposition is reached at lower temperatures (20% at 125°C). In this series, carbonyl sulfide was about 20% of the gas mixture at all temperatures and time intervals. Thiol D.S. is expressed as equivalents of carbonyl sulfide/anhydroglucose unit (AGU). Figure 4 indicates the influence the D.S. has upon decomposition at 150°C. Although increasing decomposition with increasing D.S. is suggested, results were somewhat erratic and difficult to reproduce. In this series, the gas mixture was 30% carbonyl sulfide at D.S. 0.45 and 40% at D.S. 0.60. Various salts and moisture contents had considerable influence upon the amount of decomposition and extent of thiolation at a particular temperature, which may in part explain the erratic results. Figure 5 shows the influences that

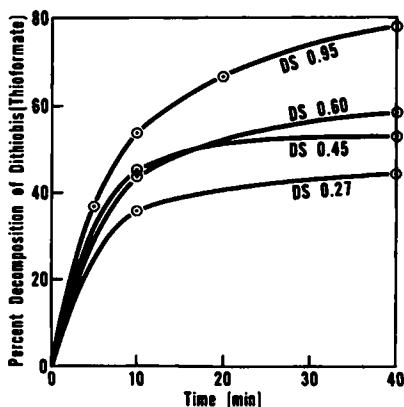


Fig. 4. How D.S. affects decomposition of starch dithiobis(thioformate) at 150°C.

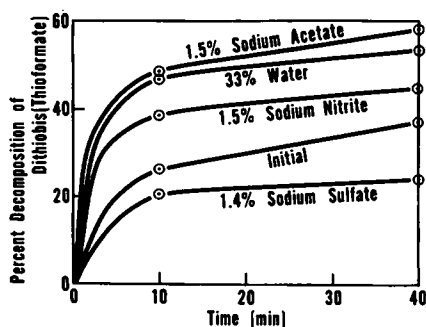


Fig. 5. Comparative effects of salts and water upon decomposition of starch.

substances have which are possibly occluded in the starch dithiobis-(thioformates) during preparation. Sodium sulfate retards the transformation to dithiocarbonate, whereas sodium acetate, sodium nitrite, and water accelerate it. About 30% carbonyl sulfide is obtained with the added salts and about 40% with water.

A completely substituted starch dithiobis(thioformate) pyrolyzed at 220°C for 20 min was saponified and found to have an intrinsic viscosity near 0.3 (intrinsic viscosity of the parent starch was 1.55). This pyrolyzed product was dark, compared to nearly colorless ones obtained from pyrolysis of lower D.S. dithiobis(thioformates) at lower temperatures.

Cellulose, dextran, and xylan dithiobis(thioformates) were pyrolyzed under various conditions. During pyrolysis of each, carbonyl sulfide and carbon disulfide evolved. The formation of carbonyl sulfide with the xylan and dextran derivatives has special significance since these polysaccharides contain almost exclusively secondary hydroxyls.

The discovery that polysaccharides can be thiolated by pyrolysis of dithiobis(thioformates) was unexpected, especially since Polyakov and associates¹³ had reported that an unsaturated cellulose formed when cellulose dithiobis(thioformate) was pyrolyzed. Furthermore, when starch dithiobis(thioformate) was heated in pyridine as in the preparation of several thio sugars, little thiolation was achieved as indicated by the small amount of carbonyl sulfide detected. An IR spectrum of the resulting product showed thionocarbonate, suggesting this pathway to be almost the exclusive one.

At present, we do not know the extent of thiolation at primary versus secondary positions in starch, nor do we know whether thiolation at secondary positions occurs with retention or inversion of configuration. Further studies are in progress with appropriate model compounds to elucidate the mechanism of this thiolation reaction.

EXPERIMENTAL

General

Pearl corn starch, cotton cellulose, and dextran were commercial grades. Xylan was prepared according to a published procedure.¹⁴ Dextran of

molecular weight between 15,000 and 20,000 was purchased from Pharmachem Corp., Bethlehem, Pa. IR spectra were recorded for solutions and films with a Perkin-Elmer Model 137 spectrophotometer. UV spectra were determined with a Perkin-Elmer Model 202 spectrophotometer. NMR spectra were recorded with a Varian HA-100 NMR spectrometer with chloroform-*d* and tetramethylsilane (τ 10.00) as the internal reference standard. Intrinsic viscosities were determined in 1*N* potassium hydroxide at 25°C with Ostwald-Cannon-Fenske viscometers.¹⁵

Preparation and Pyrolysis of Starch Dithiobis(thioformate)

Starch xanthates of varying D.S. were prepared by treating aqueous slurries of starch with potassium hydroxide and carbon disulfide and were oxidized to dithiobis(thioformates) with sodium nitrite. For example, starch (5.0 g) was slurried with water (50 ml) and potassium hydroxide (20 g) added with rapid agitation to form a smooth paste. The mixture was cooled and carbon disulfide (20 ml) was stirred in. The mixture was agitated slowly 3 hr at room temperature and diluted with ice water to 500 ml. After sodium nitrite (13 g) was added, the solution was flushed with nitrogen and stirred while 30% acetic acid (55 ml) and sulfuric acid (2*N*, 500 ml) were slowly added. A precipitate was filtered, washed with water, acetone, carbon disulfide, and hexane. Yield upon air drying: 8.9 g. Calcd for per dithiobis(thioformate) (C₉H₇O₆S₆)_x: S, 49.6%. Found: 48.9%. The IR spectrum (Nujol mull) of this product showed strong absorption near 8.1 and 9.6 μ m typical of dithiobis(thioformates), and no absorption for hydroxyl.

For starch dithiobis(thioformate) analyses, samples were treated with excess *p*-chlorothiophenol in pyridine for 15 min and washed with chloroform and hexane. Dithiobis(thioformate) content was determined from the difference in sulfur values before and after this treatment.

Pyrolyses were conducted in flasks placed in an oil bath. Flasks were equipped with stirrer and both gas inlet and outlet tubes. During pyrolysis, a slow stream of nitrogen was passed into the flask and out through a calcium chloride tube into a solution of 1% piperidine in hexane cooled to 5°C. The temperature of the material pyrolyzed reached the temperature of the bath within 5 min, which was varied from 125° to 220°C controlled to \pm 5°. The solid that formed in the piperidine-hexane solution was filtered and weighed to determine the total of carbonyl sulfide and carbon disulfide evolved. The ratio of the two gases was determined from IR spectra of the solid dissolved in chloroform.⁹

A typical example of pyrolysis is the decomposition of starch dithiobis(thioformate) (5.0 g, 48.9% sulfur) for 90 min at 200°C. The piperidine complex (3.5 g) showed carbonyl sulfide:carbon disulfide (1:1) and indicated 77% decomposition. Carbonyl sulfide corresponded to 0.58 equivalents/AGU.

Saponification

Pyrolyzates were treated with base to decompose thionocarbonate, dithiocarbonate, and unreacted dithiobis(thioformate). The pyrolyzate (1.0 g) from dithiobis(thioformate) of 48.9% sulfur was stirred with 2% sodium hydroxide 30 min at room temperature, neutralized with acetic acid (20 ml), cooled, diluted with methanol (100 ml), and filtered. The product was washed with carbon disulfide and chloroform to remove elemental sulfur. Yield: 0.55 g; S, 15.5%.

Acetolysis

The saponified product (0.5 g) was mixed with acetic anhydride (20 ml), sodium acetate (0.4 g), and zinc dust (4 g) and stirred 15 min at 120°C. The mixture was poured into ice water (400 ml) and stirred several minutes until the product solidified. The liquid was decanted and the residue extracted from excess zinc with chloroform. Evaporation of the chloroform left a syrup (0.10 g; S, 6.4%) that showed two carbonyl bands in the IR at 5.7 μm (OAc) and 5.9 μm (SAc) and no band for hydroxyl. The NMR spectrum exhibited peaks near τ 7.7 (SAc) and 8.0 (OAc) in a ratio of 1:4. Thiol D.S. was 0.62 based upon peak areas, as compared with 0.60 based upon sulfur analysis.

In another example, a saponified product (0.2 g, 12.1% S) was mixed with acetic anhydride (20 ml), zinc dust (1.0 g), and hydrochloric acid (2 ml, 10*N*) and stirred 5 min at 120°C. The mixture was poured into ice water (200 ml), stirred several minutes, and filtered. The precipitate was air dried to yield 0.2 g. Thiol D.S. was 0.44 based upon the NMR spectrum and 0.47 according to sulfur analysis.

Colorimetric Method for Thiol Determination

Starch dithiobis(thioformate) (48.9% S) was heated 30 min at 190°C and saponified as described previously. The product was treated with sodium hydroxide (12.5*N*, 1.6 ml), diluted with water (50 ml), and adjusted to pH 8 with phosphoric acid (85%, 0.8 ml). Ellman's reagent¹² (0.5 ml), prepared from 5,5'-dithiobis(2-nitrobenzoic acid) (198 mg) in disodium phosphate (0.1*M*, 50 ml), was added and the solution diluted to 100 ml. After 5 min, the optical density was determined at 420 nm. The extinction coefficient of the reagent averaged 10,670 under these experimental conditions using 4-chlorothiophenol and 2-mercaptoethanol as standards. Thiol D.S. was determined according to

$$\text{D.S.} = A/10,670 \times 18/w$$

where A = optical density and w = sample weight in grams. The thiol D.S. determined by this method (0.14) was lower than the thiol D.S. determined from sulfur analysis of the acetolysis product (0.21). The discrepancy may be due to the tendency of thiol to oxidize to disulfide.

When the alkaline solution of the thiol was treated with aluminum foil before colorimetric determination, thiol D.S. was 0.23.

Reaction Variables in Preparing Thiolated Products from Starch

Temperature. Starch dithiobis(thioformate) was prepared by treating starch (10 g) in water (100 ml) with potassium hydroxide (10 g) and carbon disulfide (40 ml) 3 hr, as previously described. Sulfur analysis of the nitrite coupled product indicated a xanthate D.S. of 0.95. Samples (1–3 g) were pyrolyzed at 125°, 130°, 150°, and 175°C, and the piperidine complexes were weighed at intervals.

D.S. Dithiobis(thioformates) derived from xanthate of D.S. 0.27, 0.45, and 0.60 were prepared by varying the ratios of potassium hydroxide to starch in the general procedure. Samples were pyrolyzed at the same temperature (150°C).

Salts and Water. To dithiobis(thioformate) (1.0 g, D.S. 0.26), sodium-sulfate (14 mg) was added. To other samples, sodium nitrite (15 mg), sodium acetate (15 mg), or water (500 mg) was added, respectively. Pyrolyses were conducted at 150°C.

Pyridine. A dithiobis(thioformate) of D.S. 1.25 was suspended in pyridine (72.5 ml) and stirred for 3.5 hr at 65°C. The piperidine complex (7.8 g) indicated 1:9 carbonyl sulfide:carbon disulfide and 62% decomposition. Sulfur (2.5 g) was extracted from the pyrolyzate with carbon disulfide and chloroform. The IR spectrum (Nujol mull) of the product showed thionocarbonate (7.8 μm).

Preparation and Pyrolysis of Other Polysaccharide Dithiobis(thioformates)

Xylan. Xylan (2.5 g) was dispersed in water (25 ml) treated with potassium hydroxide (2.5 g) and carbon disulfide (10 ml). The mixture was kept for 3 hr and oxidized with sodium nitrite (3.25 g). The yield of the dithiobis(thioformate) was 3.2 g, S, 20.4%. Pyrolysis was done in the dry state at 150°C for 40 min. The piperidine complex indicated 34% decomposition with 3:7 carbonyl sulfide:carbon disulfide.

Dextran. To a solution of dextran (5 g) in water (50 ml), potassium hydroxide (25 g) and carbon disulfide (15 ml) were added. The mixture was kept for 4 hr at 25°C, diluted with ice water (to 1 liter), then treated with sodium nitrite (12 g), and acidified with acetic acid (30 ml), followed with sulfuric acid (1*M*, 200 ml). The dextran dithiobis(thioformate), which precipitated, was filtered, washed with water followed by acetone, and air dried. Yield: 70 g; S, 17.7%.

Pyrolysis was carried out for 70 min at 150°C. The piperidine complex indicated 36% decomposition and a ratio of carbonyl sulfide:carbon disulfide evolved of 3:7.

Cellulose. Cotton (1.5 g, 40 mesh) was suspended in water (15 ml) and treated with potassium hydroxide (3 g) and carbon disulfide (10 ml). The mixture after being kept for 3 hr with occasional stirring was diluted with

water (to 200 ml) and treated with sodium nitrite (4 g). Upon acidification, the dithiobis(thioformate) precipitated and was filtered, washed with water and acetone, and air dried. Yield: 2.6 g; S, 28.9%.

The pyrolysis was done at 150°C for 40 min. The ratio of carbonyl sulfide:carbon disulfide evolved was 5:4, and decomposition was 24%.

In another preparation of the dithiobis(thioformate), cellulose (5 g, 40 mesh) was dispersed in water (50 ml) and treated with potassium hydroxide (5 g) and carbon disulfide (20 ml). The mixture was kept for 3 hr and oxidized with sodium nitrite (6.5 g). The dithiobis(thioformate) was isolated as above to yield 6.1 g, S, 10.9%. Pyrolysis was carried out in the dry state at 150°C for 40 min. The piperidine complex indicated 100% decomposition with a 1:4 ratio of carbonyl sulfide:carbon disulfide.

The authors thank C. A. Glass, D. Weisleder, and L. W. Tjarks for NMR spectra.

The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

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Received September 21, 1972