(Shear) Duggar, the causal agent of cotton root rot, the presence of glycogen was noted in aqueous extracts of sclerotia produced in soil cultures[3] of the fungus. However, neither isolation nor quantitative determination was attempted and identification was based upon color tests.

Subsequent investigations of the carbohydrate reserves of sclerotia and their utilization during germination necessitated a more thorough study of glycogen than was first reported. These results revealed that the glycogen of sclerotia, based upon solubility in hot water, is present in two forms: (a) free glycogen, which is readily extracted with hot water and (b) bound glycogen, insoluble in hot water but soluble after treatment with hot 35% potassium hydroxide. Presumably the latter form exists in chemical union with protein or some cell wall constituent and is liberated by the action of strong alkali. The fact that Tsai[4] was also able to differentiate two fractions of glycogen, free and bound, from liver and muscle and that Ling, *et al.*,[5] reported a similar finding in yeast glycogen, based upon water solubility, suggests that coexistence of labile and less labile forms of glycogen may be more or less general.

The purpose of this communication is to report the isolation, yield, and chemical properties of glycogen from sclerotia of *Phymatotrichum omnivorum*.

Experimental

Both fresh and oven-dry sclerotia were used for the isolation of glycogen but the former required less methodology in the final steps of purification.

(a) **Free Glycogen.**—Thirty grams of fresh sclerotia (the equivalent of 10.9 g. of oven-dry sclerotia) from 30-day-old soil cultures of *P. omnivorum* were killed in boiling 95% ethanol, dried at 75° and the ground material Soxhlet-extracted with 80% ethanol until complete removal of alcohol-soluble substances (16–20% of the dry weight) was effected. The dried residue from the alcohol extraction was extracted with successive portions of hot water until the test for glycogen, a reddish brown color with dilute iodine solution, was negative. The combined aqueous extracts were acidified with acetic acid, evaporated to a volume of approximately 50 ml. and the precipitated protein removed by centrifugation. Glycogen was precipitated from the opalescent solution by the addition of two volumes of 95% ethanol and purified by alternate dissolution in water and precipitation with alcohol twice. The final product, after washing with acetone and drying, was colorless, weighed 1.1 g., and was equivalent to 10.1% of the dry weight of sclerotia.

(b) **Bound Glycogen.**—The residue from (a) was suspended in 30 ml. of a 35% solution of potassium hydroxide and refluxed for two hours in boiling water. During this period of digestion most of the protein was hydrolyzed and the liberated glycogen dissolved. The insoluble cellular materials were removed from the cold alkaline solution by centrifugation and the crude glycogen was precipitated with two volumes of 95% ethanol. The first step in its purification was dissolution in water, acidification with acetic acid, and boiling to precipitate any remaining protein. After removal of the precipitate, the glycogen was again precipitated and carried through two to three series of alcohol precipitations as in (a). The final product was washed with acetone and dried at 100°. The yield of glycogen from the bound fraction was 2.9 g. and was equivalent to 26.6% of the oven-dry weight of sclerotia.

Because of the variable fresh weight of sclerotia, which were separated from the soil cultures by washing, the yields of glycogen expressed on the dry weight basis are more accurate than as a percentage of the fresh weight. The combined yields of the two forms of glycogen was 4.0 g., equal to 36.7% of oven-dried sclerotia.

Chemical Properties

No differences were found in the chemical properties of glycogen from the two fractions. The following data were obtained on glycogen from the bound fraction.

The rate of enzymatic hydrolysis of sclerotia glycogen with Taka Diastase was lower than that of either potato starch or dextrin, a characteristic property of glycogen according to Glock[6] and Morris, *et al.*[7] The latter isolated glycogen from the seed of *Zea Mays*, Golden Bantam variety. Aqueous solutions of glycogen were strongly opalescent by reflected light and were colored reddish brown by a dilute iodine solution. The color was intensified by addition of sodium chloride. Hydrolysis of 0.2057 g. of the glycogen in 2.5% hydrochloric acid for three hours at 100° gave 0.2263 g. of glucose, the equivalent of 0.2037 g. of glycogen. Glucosazone, prepared from the hydrolysate of another sample of glycogen, melted at 205–206° after recrystallization from 50% ethanol. The specific optical rotation[8] of sclerotia glycogen was 199° in aqueous solution with sodium D light. With cupric chloride[7] the glycogen preparation gave a crystallization pattern similar to that obtained with a sample of glycogen from Eastman Kodak Company, but quite dissimilar to the pattern formed when dextrin (Pfanstiehl product) was substituted for glycogen. The ash content of the sclerotia glycogen was 0.3%.

(3) Dunlap, *Am. J. Botany*, **28**, 945–947 (1941).

(4) Tsai, *Chinese J. Physiol.*, **11**, 81–93 (1937).

(5) Ling, *et al.*, *J. Inst. Brewing*, **31**, 316–321 (1925).

(6) Glock, *Biochem. J.*, **30**, 1386–1396 (1936).

(7) Morris, *et al.*, *J. Biol. Chem.*, **130**, 535–544 (1939).

(8) The author wishes to express his appreciation to Dr. James G. Potter and Dr. O. W. Silvey of the Department of Physics, Agricultural and Mechanical College of Texas, for the determination of the optical rotation.

BUREAU OF PLANT INDUSTRY
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o-Nitrophenyl Acetate

BY LUCAS C. GALATIS

A method, more convenient and efficient than previous ones,[1] for the preparation of *o*-nitrophenyl acetate is the following.

A mixture of equivalent quantities of *o*-nitrophenol and acetic anhydride is treated with one drop of sulfuric acid and then heated for three hours on the steam-bath. After the reaction mixture has cooled, it is poured dropwise and with good stirring into 200 cc. of cold water seeded with some nitrophenyl acetate. The light-yellow precipitate is filtered, washed with cold water, dried in air and stored in a vacuum desiccator over sulfuric acid. After two days the nitrophenol acetate is free of unreacted nitrophenol, and the use of alkaline reagents for removing starting material is thus obviated. The completion of this purification can be checked by the gradual rise of the melting point to 36–38°. The yield is 93% of the theoretical. The acetate can be recrystallized by dissolving it in an equal quantity of alcohol at room temperature and cooling to 0°.

(1) Lindemann and Romanoff, *J. prakt. Chem.*, **122**, 227 (1929); Brown, THIS JOURNAL, **68**, 873 (1946).

o-Nitrophenyl acetate is not attacked by boiling water and is slightly volatile with steam.

CHEMICAL LABORATORY OF THE ADMIRALTY
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2-Amino-5-thiazolesulfonic Acid Derivatives

BY H. ELDRIDGE FAITH

For the purpose of bacteriological studies several 2-amino-5-thiazolesulfonic acid derivatives have been made.[1] The intermediate compound used in synthesizing these derivatives was 2-acetamino-5-thiazolesulfonyl chloride, made in 15 to 25% yield by the action of chlorosulfonic acid on 2-acetaminothiazole. The sulfonyl chloride reacted smoothly with several amines in pyridine to form the corresponding 2-acetamino-5-thiazolesulfonamides which were deacetylated by acid hydrolysis. When stirred with sodium sulfite solution, 2-acetamino-5-thiazolesulfonyl chloride was reduced to 2-acetamino-5-mercaptothiazole (VI).

TABLE I

2-AMINO-5-THIAZOLESULFONIC ACID DERIVATIVES

Compound	Name	Decompn. p., °C. (uncor.)	Yield,[a] %	Analytical data,[b] % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
I	2-Acetamino-5-thiazolesulfonamide	273	52.5	27.12	3.19	18.98	27.12	3.18	18.93
II	2-(2-Amino-5-thiazolesulfonamido)-pyridine	228	65	37.57	3.14	21.86	37.61	3.11	22.13
III	2-(2-Amino-5-thiazolesulfonamido)-pyrimidine	253	39	32.68	2.74	27.22	32.80	2.73	27.29
IV	2-(2-Amino-5-thiazolesulfonamido)-thiazole	235	45	27.47	2.30	21.36	27.37	2.23	21.15
V	*p*-(2-Amino-5-thiazolesulfonamido)-aniline[c]	196	57	39.99	3.73	20.73	39.88	3.81	20.66
VI	2-Acetamino-5-mercaptothiazole	203	73.5	34.46	3.47	16.08	34.37	3.09	15.98

[a] Based on the amount of 2-acetamino-5-thiazolesulfonyl chloride employed. [b] The micro-analyses were performed by Dr. Carl Tiedcke. [c] The intermediate amine used was *p*-aminoacetanilide.

Experimental

2-Amino-5-thiazolesulfonic Acid Derivatives

2-Acetamino-5-thiazolesulfonyl Chloride.—A 15-g. (0.106 mole) portion of 2-acetaminothiazole[2] was heated with 61 g. (0.53 mole) of chlorosulfonic acid at 100° for two hours and fifteen minutes.[3] Then the solution was poured onto 560 g. of ice causing a precipitate to form. The precipitate was filtered off, washed with ice water and dried over sodium hydroxide at reduced pressure. The product weighed 6.2 g. and was used in subsequent reactions without further purification. It decomposed at 220° when inserted in a bath at 200° and decomposed at the same point after crystallizing from acetone. A positive test for chlorine and elemental analyses of the amide derived from the compound indicated that the compound was a 2-acetaminothiazolesulfonyl chloride. Several thiazole studies[4,5,6] have given evidence that the hydrogen in position 5 of a compound like 2-acetaminothiazole would be more active than the one in position 4. This evidence indicated that the sulfonyl chloride was probably in position 5 on the thiazole nucleus.

2-Acetamino-5-thiazolesulfonamide (I).—This derivative was prepared from 5.39 g. of 2-acetamino-5-thiazolesulfonyl chloride in acetone by introducing ammonia with cooling. A yield of 2.6 g. of 2-acetamino-5-thiazolesulfonamide (I) was obtained after crystallizing from dilute ethanol. The sulfonamide group of this compound hydrolyzed rapidly to the sulfonic acid group in the presence of hydrochloric acid or in a solution of hydrogen chloride in 95% ethanol at various concentrations. No conditions were found for selectively hydrolyzing the acetyl group without affecting the sulfonamide portion. No 2-amino-5-thiazolesulfonamide was isolated under conditions of partial hydrolysis of 2-acetamino-5-thiazolesulfonamide.[7]

2-Amino-5-thiazolesulfonamides.—The N^5 substituted sulfonamides (II, III, IV and V) were readily made by heating 2-acetamino-5-thiazolesulfonyl chloride at 60° for one and one-half hours with the appropriate amine in dry pyridine. The pyridine solution was then diluted with water, neutralized with dilute sodium hydroxide and vacuum distilled. The residual acetamino derivative was dissolved in dilute sodium hydroxide solution to remove any alkali-insoluble material, and was then heated with 10% hydrochloric acid to remove the acetyl group. 2-(2-Acetamino-5-sulfonamido)-thiazole (IV) became dark and produced a sulfide odor when subjected to a hydrolysis in hot 2.5 *N* sodium hydroxide. The 2-amino-5-thiazolesulfonamides were purified by crystallization from dilute ethanol.

2-Acetamino-5-mercaptothiazole (VI).—By the procedure used in reducing *p*-acetaminobenzenesulfonyl chloride to *p*-acetaminobenzenesulfinic acid with sodium sulfite,[8] a 73.5% yield of 2-acetamino-5-mercaptothiazole was obtained from 2-acetamino-5-thiazolesulfonyl chloride. Evidently any 2-acetamino-5-thiazolesulfinic acid formed was reduced immediately to the mercapto derivative. Zinc dust in 95% ethanol at 10° also accomplished this reduction. The compound was soluble in dilute potassium hydroxide and was purified by crystallizing from hot water.

(1) After this work was completed Backer and Buisman published on a similar work which included a description of compounds I, II and IV. *Rec. trav. chim.*, **63**, 228 (1944); *C. A.*, **40**, 2446 (1946).

(2) Jensen and Thornsteinsson, *Dansk Tids. Farm.*, **15**, 41 (1941); *C. A.*, **35**, 5109 (1941).

(3) Heating sodium 2-acetamino-5-thiazolesulfonate with chlorosulfonic acid or with phosphorus pentachloride was not as satisfactory a method of producing the sulfonyl chloride.

(4) Ochiai and Nagazawa, *Ber.*, **72**, 1470 (1939).

(5) Backer and Buisman, *Rec. trav. chim.*, **63**, 226 (1944); *C. A.*, **40**, 2446 (1946).

(6) Erlenmeyer and Kiefer, *Helv. Chim. Acta*, **28**, 985 (1945); *C. A.*, **40**, 1500 (1946).

(7) Since this work was done, Backer and Buisman reported obtaining 2-amino-5-thiazolesulfonamide, *Rec. trav. chim.*, **63**, 228 (1944).

(8) "Organic Syntheses," Coll. Vol. I, p. 7.

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Strength of Aqueous Thiocyanic Acid

BY MEL GORMAN AND JOSEPH CONNELL[1]

In the course of some work on the thiocyanates it became necessary to know the strength of

(1) Present address: American Can Company, San Francisco, California.