Acknowledgment.—The author is indebted to The Research Corporation for support of this research.

Experimental

A mixture of 45.9 g. (0.26 mole) of NBS, 27.1 g. (0.33 mole) of cyclohexene (found by iodometric titration to contain 0.05 mole of cyclohexene hydroperoxide per liter), and 185 ml. of C. P. anhydrous benzene was stirred and brought to reflux in twenty-six minutes; six minutes later the NBS was shown by testing with potassium iodide to be completely consumed. After cooling and filtering off succinimide, the filtrate was fractionally distilled; in addition to solvent and unchanged cyclohexene, 21.0 g. (50.3%) of 3-bromocyclohexene, b. p. 45–47° at 10 mm. and 6.7 g. of a colorless oil, b. p. 51–57° at 1 mm.,[5] were obtained. Careful fractional recrystallization of solids which separated from the residues left after removal of each of the above liquid fractions gave a total of 0.44 g. (1%, based on NBS) of N-phenylsuccinimide, m. p. 155.2–155.7° (cor.) unchanged by admixture with an authentic sample.

(5) On long standing at 0°, this oil deposited 1.31 g. of massive colorless crystalline material, tentatively identified as 3,6-dibromocyclohexene[1] (m. p. 107.5–108.3° (cor.). *Anal.* Calcd. for $C_6H_8Br_2$: C, 30.03; H, 3.36. Found: C, 30.43; H, 3.61); the residual oil was shown to consist of at least 60% 1,2-dibromocyclohexane (b. p., m. p., mixed m. p. with an authentic sample; separated from reactive unsaturated dibromides by virtue of its inertness toward trimethylamine at room temperature).

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The Bromine-Sensitized Photochemical Formation of Hexabromoethane from Carbon Tetrabromide

BY L. B. SEELY, JR., AND J. E. WILLARD

When very dilute solutions (10^{-3} *M*) of bromine in purified and degassed liquid carbon tetrabromide were illuminated at 95° with light of wave length between 4100 and 4500 Å., more bromine was produced. We have assumed that this was due to the formation of hexabromoethane from two molecules of carbon tetrabromide with the consequent liberation of one molecule of bromine.

Our interest in this reaction was occasioned by the simplicity and symmetry of the reacting molecules, the unusual nature of the process as characterized by the formation of a carbon-to-carbon bond, the relation of this reaction to other reactions involving an attack by a light-activated halogen on a halogen–carbon bond and, lastly, the possibility of tracer studies as a means of investigating the mechanism.

Precise studies of this reaction have been carried out on carbon tetrachloride solutions. The carbon tetrachloride was purified according to procedures previously described.[1] The carbon tetrabromide was purified by repeated recrystallizations from carbon tetrachloride. The bromine was distilled in vacuum from anhydrous calcium bromide and sealed in small bulbs for later introduction into the reaction mixture. The mixture of tetrahalides was purified and degassed by flushing with pure nitrogen, evacuating while frozen and distilling several times in vacuum.

(1) Dickinson and Leermakers, THIS JOURNAL, **54**, 3853 (1932).

At 0.5 *M* carbon tetrabromide, 2×10^{-3} *M* bromine and 50° the reaction proceeded with a quantum yield of about 1.0×10^{-3} mole of bromine per einstein of light absorbed. Under other conditions quantum yields between 10^{-4} and 10^{-2} were recorded. An increase in the bromine concentration caused a decrease in the quantum yield. The quantum yield increased with increase in the carbon tetrabromide concentration but the quantitative dependence on this concentration varied with the bromine concentration. The temperature coefficients were likewise dependent on the bromine concentration.

The explanation of this behavior appears to be the existence of two reaction mechanisms, prevailing in different bromine concentration ranges, with a transitional range between. At bromine concentrations above 3×10^{-3} *M* (0.5 *M* carbon tetrabromide, 50°) the reaction followed the rate law

$$\frac{d[C_2Br_6]}{dt} = \frac{kI_{abs}f([CBr_4])}{[Br_2]^2}$$

The experimental evidence to date indicates that $f([CBr_4])$ should be the first power. This has been hard to rationalize in terms of the usual mechanisms, since two carbon tetrabromide molecules are required to produce one hexabromoethane molecule, and since the light dependency is first power.

At bromine concentrations below 1×10^{-3} *M*, there is evidence that the rate law becomes independent of both carbon tetrabromide and bromine, following the law

$$d[C_2Br_6]/dt = k'I_{abs}$$

This is the law to be expected if the process determining the concentration of tribromomethyl free radicals is the combination of these radicals. Further studies in this region may provide experimental evidence concerning such processes.

At bromine concentrations above 1×10^{-3} *M* the quantum yield for the photo-activated exchange of free bromine with the bromine in carbon tetrabromide (determined by means of radiobromine) was very much higher than the quantum yield for the formation of hexabromoethane and was very sensitive to trace amounts of impurities.

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The Glycogen Content of *Phymatotrichum Sclerotia*[1]

BY DAVID R. ERGLE

In a recent chemical study[2] of the mycelial and sclerotial stages of *Phymatotrichum omnivorum*

(1) Published with the approval of the Director of the Texas Agricultural Experiment Station as Technical Paper No. 996.

(2) Ergle and Blank, *Phytopathology*, **37**, 153–161 (1947).

(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.

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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).