redistilled water. The oxidation was conducted in the usual manner at 18° in the dark.

Moles of formic acid produced per mole of carbohydrate were 2.4 moles in 46 hr., 2.5 moles in 110 hr., 2.8 moles in 214 hr. and 2.9 moles in 314 hr.; moles of periodate consumed in the same times were 6.8, 7.5, 8.2 and 9.0, respectively. These are consistent with the expected values of 3 moles of formic acid produced and 9 moles of periodic acid consumed.

LAFAYETTE, INDIANA

[CONTRIBUTION FROM THE CHEMICAL LABORATORY, UNIVERSITY COLLEGE, GALWAY, IRELAND]

The Action of Phenylhydrazine on the Periodate Degradation Products of β-D-Glucopyranosyl Sulfones

BY EILIS BLANCHFIELD AND THOMAS DILLON

RECEIVED SEPTEMBER 18, 1952

When the dialdehyde sirup obtained by the action of periodic acid on β-D-glucopyranosyl sulfone reacts with phenylhydrazine, the products are glyoxal diphenylhydrazone and benzenesulfinic acid. The reaction is an example of the reaction between the periodate oxidation products of glucosides and phenylhydrazine, discovered by Barry and the benzenesulfonic acid found in the products of the reaction is due to disproportionation of the benzenesulfinic acid.

In a recent paper under the above title, Bonner and Drisko[1] state that phenylhydrazine reacts with the periodate degradation products of pyranosyl sulfones to give glyoxal bisphenylhydrazone and benzenesulfonic acid, and to account for the latter product of the reaction they formulate a series of reactions in which phenylhydrazine acts as an oxidizing agent. It seemed to us that the reactions described were merely cases of the reaction discovered by Barry[2] in this Laboratory in 1943, in which phenylhydrazine disintegrates the periodate oxidation products of starch and of other 1,4-linked polysaccharides forming glyoxal bisphenylhydrazone. The reaction appears to be generally applicable to periodate-oxidized glucosides and indeed to all semi-diacetals of glyoxal[3] and, since there is no reason why the sulfones should be an exception, there is no necessity to assume oxidation by phenylhydrazine as part of the mechanism of the change and the presence of benzenesulfonic acid in the products must be otherwise explicable. As a matter of fact, it has long been known[4] that aqueous solutions of benzenesulfinic acid on heating to 130°, change into benzenesulfonic acid and phenylbenzene thiosulfonate, that this disproportionation takes place slowly at ordinary temperatures and that it is much accelerated by the presence of hydrochloric acid. We have actually observed that its *alkaline* solution develops an odor of thiophenol on standing for a few days. Bonner and Drisko appear to have experienced some difficulty in proving the presence of benzenesulfonic acid in their reaction products, and they were able to separate only a small fraction of the yield of this acid to be expected from their theory.

We repeated Bonner and Drisko's experiment with phenyl β-D-glucopyranosyl sulfone and, when we took the necessary precautions to avoid disproportionation, we had no difficulty in identifying benzenesulfinic acid among the reaction products. Without such precautions, however, the latter acid was not found. Like other periodate degradation products of glucosides,[3b] phenyl β-D-glucopyranosyl sulfone also reacted with hydroxylamine to give glyoxime. Here there could be no question of an oxidation.

Experimental

Preparation of Phenyl β-D-Glucopyranosyl Sulfone and Its Oxidation with Periodate.—These experiments were carried out by the methods of Bonner and Drisko,[5] yielding a clear sirup. This sirup dissolved in dilute sulfuric acid, and did not give with ferric chloride the orange precipitate characteristic of benzenesulfinic acid.[6]

Reaction with Phenylhydrazine.—0.03 g. of the above sirup was dissolved in 2.5 cc. of warm water and 0.03 cc. of phenylhydrazine was added, followed by sufficient glacial acetic acid to bring it into solution. The mixture was left aside for 5 days at room temperature. The color of the liquid changed from yellow to red, and crystals of glyoxal bisphenylhydrazone gradually separated. These were filtered off and the filtrate was brought to *p*H 12 and extracted 14 times with ether, to remove excess phenylhydrazine. A portion of the remaining alkaline solution was neutralized with strong hydrochloric acid and then made acid with sulfuric acid. When a concd. soln. of ferric chloride was added, the orange precipitate characteristic of benzenesulfinic acid fell out.

The rest of the alkaline solution was left aside and after about 48 hours it smelled strongly of thiophenol. A little of it acidulated and tested with ferric chloride now gave no precipitate. On further standing on a watch glass, the alkaline solution deposited crystals of sodium salts. These were decomposed with a little sulfuric acid and extracted with cold water, in which benzenesulfinic acid is sparingly soluble. The aqueous solution on evaporation deposited crystals, m.p. 44°, evidently benzenesulfonic acid. The undissolved residue was extracted with ether, in which benzenesulfinic acid is easily soluble. On evaporation, the ether solution left a minute amount of crystalline material.

One gram of the sirup was dissolved in 75 cc. of water and to the solution 1.65 cc. of phenylhydrazine added, followed by sufficient glacial acetic acid to bring it into solution. This mixture was heated on the boiling water-bath for 30 min., as in Bonner and Drisko's experiment. On cooling, crystals of glyoxal bisphenylhydrazone separated and the mixture was extracted 15 times with 20 cc. of ether each time. The extracted liquid was brought to *p*H 12 and again extracted several times with ether. It was then neutralized with hydrochloric acid, acidulated with sulfuric acid and treated with ferric chloride, whereupon a heavy orange precipitate fell out. This was filtered off, treated with excess of ammonia and filtered from ferric hydroxide. When the filtrate was acidulated with hydrochloric acid a

(1) W. A. Bonner and R. W. Drisko, THIS JOURNAL, **73**, 3701 (1951).

(2) V. C. Barry, *Nature*, **152**, 537 (1943).

(3) (a) C. Harries, *Ber.*, **36**, 1935 (1903); (b) T. Dillon, *Nature*, **155**, 546 (1945).

(4) Beilstein, "Handbuch Organ. Chem. Dritte Aufl.," Vol. 2, p. 108.

(5) W. A. Bonner and R. W. Drisko, THIS JOURNAL, **70**, 2435 (1948); **73**, 3699 (1951).

(6) T. Thomas, *J. Chem. Soc.*, **95**, 342 (1909).

crystalline precipitate formed; m.p. 85°, lit. m.p. benzenesulfinic acid 83–84° (Beilstein, ref. 4), 86–87° (Thomas[6]).

Reaction with Hydroxylamine.—An 8% soln. (approx.) of hydroxylamine was made by treating the hydrochloride dissolved in the smallest possible quantity of water with a soln. of the equivalent of sodium dissolved in abs. ethanol. Five cc. of this soln. was added to 0.02 g. of the sirup and the mixture was evaporated on the boiling water-bath. When dry, the beaker was covered with an inverted watch-glass and left on the water-bath. In a short time the watch-glass became clouded and on examination under the microscope, was seen to be covered with the characteristic crystals of sublimed glyoxime. The residue in the beaker gave the ferric chloride test for benzenesulfinic acid.

Disproportionation of Benzenesulfinic Acid in Alkaline Solution.—Benzenesulfinic acid was prepared by the method of Thomas.[6] The sample had m.p. 83–84°. An aqueous solution was made alkaline with caustic soda and left aside. After a few days the solution had a smell of thiophenol, which increased considerably on warming.

GALWAY, IRELAND

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY AND THE FOREST PRODUCTS LABORATORY, UNIVERSITY OF CALIFORNIA]

On the Cyclitols Present in Sugar Pine (*Pinus lambertiana* Dougl.)

BY CLINTON E. BALLOU[1] AND ARTHUR B. ANDERSON[2]

A water extract of sugar pine heartwood has been shown to contain myoinositol, *d*-inositol and sequoyitol in addition to the previously reported pinitol. Myo-inositol was found to be present in the aqueous sapwood extract along with pinitol. A procedure of fractional acetonation was employed to separate the cyclitol mixture and led to isolation of the pure compounds. Filter paper chromatography was applied to the separation and identification of four cyclitols, and three cyclitol derivatives.

The constituents of the heartwood of sugar pine and other members of the genus *Pinus* have been investigated systematically by Erdtman, Lindstedt and others in an effort to obtain evidence for a classification of these plants on the basis of the chemical composition of the heartwood extractives.[3] In addition to the large number of phenolic substances identified, these workers have commented on the general occurrence of pinitol[4] in the heartwood of *Haploxylon* pines (five needled). Although chromatographic techniques were employed, no other cyclitols were identified.[5] Erdtman, who was unable to isolate pinitol from the sapwood of the Haploxylon pine *Pinus strobus*, suggested that pinitol is a true heartwood substance.[6] One of us,[7] however, has recently shown pinitol to be present in the sapwood of the sugar pine.

The work reported below was undertaken to clarify the facts concerning the composition and distribution of the cyclitol fraction in sugar pine heartwood and sapwood. Re-examination of the water-soluble constituents of the heartwood of the sugar pine led to the detection and subsequent isolation of the cyclitols myoinositol, *d*-inositol and sequoyitol,[8] in addition to pinitol. The presence of pinitol and myo-inositol in the sapwood of sugar pine has been confirmed by paper chromatography, and a method for their quantitative estimation in wood extracts has been devised.

In conjunction with the chemical separations, we have applied the paper-strip chromatographic technique to the separation of the four cyclitols. myo-inositol, *d*-inositol, allo-inositol and muco-inositol, and the cyclitol derivatives, pinitol, sequoyitol and quebrachitol. That they may be readily separated by chromatography is in contrast to the difficulty encountered in separating the hexitols by this method.[9]

Isolation of the individual cyclitols from the heartwood extract was effected by a procedure of fractional acetonation. With sulfuric acid as the catalyst, pinitol and *d*-inositol were acetonated to acetone-soluble isopropylidene compounds, while the unreacted cyclitols, sequoyitol and myo-inositol, remained undissolved. Part of the sequoyitol was then isolated from this insoluble residue by fractional crystallization from water.[8] The sequoyitol remaining in the mixture with myo-inositol was removed by acetonation with zinc chloride–glacial acetic acid catalyst,[10] which left most of the myo-inositol undissolved[11] and in a form easily purified. *d*-Inositol was isolated by column chromatography from the mother liquors left after crystallization of the diisopropylidene pinitol.

Experimental

Preparation of Wood Extracts.—Air-dried sugar pine sawdust (500 g.) was introduced into a 4-liter glass percolator and extracted three times with water using the airlift extraction method.[12] The extracts were combined and concentrated to 100 ml. *in vacuo*. This concentrate was cooled and centrifuged to remove water insolubles that had precipitated during concentration. The supernatant was then poured slowly, with vigorous stirring in 300 ml. of 95% ethanol to precipitate the gums that were present. The precipitate was removed by centrifugation, and the resulting clear solution was used in the following studies. It will be called the "water extract."

Chromatography of Cyclitols and Sugar Pine Extracts.—Various procedures have been described for the chroma-

(1) Public Health Service Research Fellow of the National Heart Institute.

(2) Forest Products Laboratory, University of California.

(3) For reviews see: H. Erdtman, *Svensk. Chem. Tid.*, **63**, 43 (1951); G. Lindstedt, *Acta Chem. Scand.*, **5**, 129 (1951).

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(5) G. Lindstedt and A. Misiorny, *Acta Chem. Scand.*, **5**, 121 (1951).

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