[CONTRIBUTION FROM THE RESEARCH AND DEVELOPMENT DEPARTMENT, U. S. NAVAL POWDER FACTORY]

Effect of Aqueous Sulfuric Acid on Sugars. III. Isolation and Chemistry of a D-Arabinopyranosyl-D-arabinopyranoside Obtained from D-Arabinose[1,2]

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D-Arabinose was treated at room temperature with 20 *N* sulfuric acid and the resulting products acetylated by adding an excess of acetic anhydride to the acid solution. It was found that approximately 1% of the acetylated products were adsorbed from a chloroform solution by a mixture of silicic acid and Celite. When the adsorbed material was eluted with acetone a sirup was obtained which was crystallized from a mixture of toluene and ether, m.p. 116°, $[\alpha]_D$ +21°. Analysis indicated that the compound was a hexa-*o*-acetylpentobiose. Deacetylation yielded a non-reducing pentobiose which gave only D-arabinose on hydrolysis. The compound would seem to consist of two D-arabinose units linked through carbons one. On treatment with sodium metaperiodate, one mole of pentobiose consumed 4 moles of periodate and liberated 2 moles of formic acid. This is in agreement with the formulation of the compound as D-arabinopyranosyl-D-arabinopyranoside. The low optical rotation suggests the α,α-configuration.

A study of the development of the ultraviolet absorption spectra[3] of solutions of aldoses in aqueous sulfuric acid has shown that the ultraviolet absorption which develops is primarily due to the formation of certain compounds which can be extracted from aqueous acid solution by means of ether. The same ether-soluble compounds are formed irrespective of either the acid concentration or the temperature. It was also found that the ultraviolet absorption of a solution of an aldose in aqueous sulfuric acid reaches a steady state which is dependent on the concentration of the acid, the temperature, the configuration and the concentration of the sugar. A probable reason for the observed steady state is that at this point the rate of formation of the ether-soluble compounds with ultraviolet absorption characteristics is equal to the rate at which these same compounds are removed by polymerization to form insoluble products. After the steady state has been reached, the concentration of the intermediate compounds formed during the transformation of the aldose into compounds with ultraviolet absorption characteristics should be at a maximum. It was thought that acetylation after the steady state had been reached might form stable derivatives of any unstable intermediates and hence permit them to be isolated and identified. Although the isolation and identification of such compounds postulated by Isbell[4] or Wolfrom and co-workers[5] as intermediates in the degradation of aldoses in acid media would be necessary for a complete understanding of the reaction, it was considered that any compounds which could be isolated would assist in understanding the action of acid on the sugars. We wish to report the isolation and identification of a D-arabinopyranosyl-D-arabinopyranoside formed by the action of sulfuric acid on D-arabinose.

D-Arabinose was dissolved in 20 *N* sulfuric acid and the solution continuously extracted with ether in order to minimize the reaction of the ether-extractable compounds either with themselves or their precursors. At the end of 24 hours, the aqueous acid phase was cooled to −10° and acetylated by the addition of 10–15 volumes of acetic anhydride. After standing at room temperature for 24 hours, the mixed acetates were isolated by pouring the acetylation mixture over ice and sodium acetate and extracting the acetates with chloroform. When the chloroform solution containing approximately 150 g. of the mixed acetates was filtered through silicic acid[6]–Celite[7] on a buchner funnel, approximately 2 g. of crude material was retained on the adsorbent. This adsorbed material could not be removed from the adsorbent by repeated washing with chloroform (U.S.P.). Elution of the adsorbent with acetone yielded a sirup. The sirup, which was insoluble in ether, was crystallized from a mixture of toluene and ether. Elemental analyses indicated a hexa-*o*-acetylpentobiose. Deacetylation with barium methoxide yielded a compound which gave the correct analysis for a pentobiose monohydrate. The latter compound gave no reduction of Fehling solution or potassium ferricyanide by the Hagedorn–Jensen[8] method of analysis. After hydrolysis with 1 *N* hydrochloric acid, however, the reducing power was that of D-arabinose. The hydrolyzed product also had the optical rotation of D-arabinose and gave the bromophenylhydrazone of D-arabinose on treatment with bromophenylhydrazine and sodium acetate.

One can conclude that the compound consists of two D-arabinose units linked through their reducing groups.

That the pentobiose is a pyranoside is indicated by the results of periodate oxidation in which one mole of the pentobiose consumed 4 moles of periodate and liberated two moles of formic acid.

Hudson[9] has calculated the specific optical rotation of a hypothetical β,β,L-arabinopyranosyl-L-arabinopyranoside to be +264°. The low specific rotation of the pentobiose reported here would therefore suggest the α,α-linkage. It is of interest that Vogel[10] has reported a non-reducing disaccharide which he prepared by heating L-arabinosan with zinc chloride. Its constants were: $[\alpha]_D$ +18.9°, m.p. 153–55°. It is possible that Vogel's compound, which is of the L-series, corresponds to

(1) Published with permission of the Bureau of Ordnance, Navy Department. The opinions and conclusions are those of the author.

(2) Abstracts, Papers Am. Chem. Soc., **129**, 7D (1956).

(3) F. A. H. Rice and L. Fishbein, THIS JOURNAL, **78**, 1005 (1956).

(4) H. S. Isbell, *J. Research Natl. Bur. Standards*, **32**, 45 (1944).

(5) M. L. Wolfrom, R. D. Schuetz and L. F. Cavalieri, THIS JOURNAL, **71**, 3518 (1949).

(6) Merck reagent grade obtained from Merck and Co., Rahway, N. J.

(7) No. 535 obtained from the Johns-Manville Co., New York, N. Y.

(8) H. C. Hagedorn and B. N. Jensen, *Biochem. Z.*, **135**, 46 (1923).

(9) C. S. Hudson, THIS JOURNAL, **38**, 1566 (1916).

(10) H. Vogel, *Helv. Chim. Acta*, **11**, 1210 (1928).

our compound of the D-configuration prepared from D-arabinose. The question is under investigation.

Experimental

Acetylation of D-Arabinose and the Products Formed by the Reaction of the Pentose with 20 *N* Sulfuric Acid.—Fifty grams of D-arabinose ($[\alpha]^{22}D$ −105° (H_2O, *c* 5)) was dissolved in 50 ml. of 20 *N* sulfuric acid. The solution was continuously extracted with ether for a period of 24 hours and then cooled to −10°. Redistilled acetic anhydride (500 ml.) was added over a period of two hours at such a rate that the temperature was maintained between −10 and 0°. The acetylation mixture was allowed to stand at room temperature for 24 hours and then poured over a mixture of 3 kg. of ground ice and 250 g. of anhydrous sodium acetate. After from 4 to 6 hours the mixture was extracted six times with 500-ml. portions of chloroform. The chloroform solution was dried over *anhyd.* sodium sulfate, filtered, and concentrated to dryness; yield approximately 150 g.

Separation of the Hexa-*o*-acetylpentobiose by Adsorption on a Silicic Acid–Celite Mixture.—The adsorbent, consisting of a 3:2 (by weight) mixture of silicic acid[6]–Celite[7] was packed under suction in a 9 × 7 cm. fritted glass buchner funnel. Sufficient adsorbent was used to form a layer 5 cm. thick. The mixed acetates were dissolved in 1 liter of chloroform (U.S.P.) and the solution filtered through the adsorbent under suction. The adsorbent was washed on the funnel with 2 liters of chloroform (U.S.P), and then the adsorbed material was eluted with 2 liters of acetone. The acetone extract was concentrated under reduced pressure to a thick sirup which upon trituration with ether turned into a yellow friable powder; weight approximately 2 g. Only a few milligrams of additional material was obtained when the chloroform filtrate was recycled through fresh adsorbent. The dry powder was washed several times with ether and then crystallized by first dissolving it in 4 ml. of boiling toluene and then, when the solution had cooled to room temperature, adding sufficient ether to form a slightly turbid solution. The solution was filtered, an equal volume of ether was added and the turbid solution allowed to stand for several days at 0°. The crystals were removed by filtration and repeatedly recrystallized from a mixture of toluene and ether. The compound melted at 116–117°,[11] $[\alpha]^{24}D$ + 21° ($CHCl_3$, *c* 2.2), yield approximately 500 mg.

Anal. Calcd. for $C_{22}H_{30}O_{15}$: C, 49.44; H, 5.63. Found: C, 49.03; H, 5.63. The compound was saponified at 0° with 0.1 *N* NaOH: Calcd. for a hexa-*o*-acetylpentobiose: 11.25 ml. of 0.1 *N* NaOH per 100 mg. Found: 11.25 ml. of 0.1 *N* NaOH per 100 mg.

Deacetylation of the Hexa-*o*-acetylpentobiose with Barium Methoxide.—Five hundred milligrams of the hexa-*o*-acetylpentobiose was dissolved in 20 ml. of methyl alcohol, cooled to 0°, and 0.25 ml. of a 1.8 *N* methanolic solution of barium methoxide added. The solution was kept at 0° overnight and then after the addition of 57 mg. of oxalic acid dihydrate, the solution was concentrated to dryness at 0° under reduced pressure. The dry residue was extracted several times with boiling ether and then with boiling methyl alcohol. The methanol solution was concentrated under reduced pressure at room temperature to approximately 1 ml. and the disaccharide precipitated by the addition of ether. The compound was extremely difficult to crystallize and was usually obtained in an amorphous condition. On several occasions, however, when sufficient isopropyl alcohol was added to form a slightly turbid solution, the compound crystallized after standing several weeks at room temperature; m.p. 112–13°, $[\alpha]^{24}D$ −22° (MeOH, *c* 0.7), $[\alpha]^{23}D$ −22° (H_2O, *c* 2.8).

Anal. Calcd. for $C_{10}H_{18}O_9 \cdot H_2O$: C, 40.01; H, 6.72. Found: C, 40.12; H, 6.67.

Acid Hydrolysis of the Pentobiose.—An amount of 97.6 mg. of the pentobiose was dissolved in 5 ml. of 1 *N* HCl and heated under reflux for 4 hours. The solution was cooled and the volume adjusted to 5 ml. The optical rotation of the solution was $[\alpha]^{20}D$ −103°. This is in good agreement with the observed rotation of D-arabinose.[12] D-Arabinose when treated with 1 *N* HCl in the same manner showed $[\alpha]^{20}D$ −106.4°.

The hydrolyzed solution was treated with silver carbonate and filtered to remove the hydrochloric acid, treated with hydrogen sulfide and filtered again to remove traces of silver and then boiled for a few minutes to remove any hydrogen sulfide. The neutral solution was then treated with 180 mg. of bromophenylhydrazine hydrochloride together with 180 mg. of sodium acetate and boiled for 10–15 minutes. On cooling light yellow crystals separated (m.p. 162–163°). This crystalline material was indistinguishable by infrared analysis from the bromophenylhydrazone of D-arabinose.[13]

Anal. Calcd. for $C_{11}H_{15}O_4N_2Br$: C, 41.39; H, 4.74; N, 8.77. Found: C, 41.42; H, 4.51; N, 8.62.

Reducing Power of the Pentobiose.—The pentobiose did not reduce Fehling solution, and did not show any reducing power as determined by the Hagedorn–Jensen[8] method. After hydrolysis, however, the reducing power (Hagedorn–Jensen method) was equal to that shown by an equivalent amount of D-arabinose.

Periodate Oxidation of the Pentobiose.—The pentobiose (67.5 mg.) was dissolved in 5 ml. of water and 45 ml. of 0.2 *M* sodium metaperiodate added. Aliquots of 10 ml. were titrated with 0.1 *N* sodium arsenite solution. At the end of 8 hours one mole of the pentobiose had consumed 4.03 moles of periodate and liberated 2.1 moles of formic acid. The formic acid was determined by titration with 0.1 *N* sodium hydroxide to *p*H 7.

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(11) All melting points were taken on a Fisher–Johns melting point block and are uncorrected.

(12) O. Ruff, *Ber.*, **32**, 550 (1899).

(13) E. Fischer, *ibid.*, **27**, 2491 (1894).