5-Phospho-D-arabonic Acid

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Introduction

As a result of recent investigations of the formation (1) and degradation (2) of pentoses in nature, phosphorylated acids of the carbohydrate series have gained importance. There are reasons to believe that D-ribose 5-phosphate derived from D-glucose 6-phosphate is formed by the epimerization of an arabinose derivative. 5-phospho-D-ribonic acid is not easily accessible in large amounts since it has to be prepared from D-ribose 5-phosphate, a starting material which is, itself, difficult to obtain. 5-Phospho-D-arabonic acid, on the other hand, is easily prepared. According to the findings of Neuberg and Collatz (3) this substance can be made by simple oxidation of D-fructose 6-phosphate with molecular oxygen. The preparation can be accomplished without difficulty by the procedure described below.

Experimental

To 10 g. Ba fructose 6-phosphate, dissolved in 75 ml. water, a clear solution of 100 g. $Ba(OH)_2 \cdot 8H_2O$ in 1,100 ml. water was added. Oxygen (from a bomb) was bubbled through this solution. The oxygenation may be enhanced by constant shaking or stirring. There is no need to purify the commercially available oxygen. A finer dispersion of the gas can be obtained by attaching an inverted sintered glass funnel to the inlet tube. The oxygen was bubbled through for 3 hr. at room temperature, then 1 hr. at 30°C., and an additional hour at 40°C. The reaction will be over after this period of time or earlier, depending on the dispersion of the gas. The resulting solution no longer reduces Fehling's solution (prepared with $CuCl_2$) or does so to a very slight degree. CO_2 gas was then bubbled through, first at room temperature,

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then with warming on the water bath. The neutral barium salt of 5-phospho-D-arabonic acid separated in an easily filtered form together with $BaCO_3$. The precipitate was centrifuged or filtered off by suction and washed with water. Still damp, the mixture of barium compounds was triturated in a mortar with 200 ml. water and sufficient 50% perchloric acid to give a clear solution. Enough of a saturated aqueous solution of barium hydroxide was then added to keep the reaction just acid to Congo red. The resulting solution, where necessary after filtering, was added dropwise to the 5½-fold volume of 96% ethyl alcohol.[2] The acid barium salt precipitated in flocculent or powdery form. After a few hours it was filtered off by suction, washed with alcohol and dried in air. It was then suspended in 180 ml. water. A clear solution was obtained with a few drops $HClO_4$, and after establishing a slight acidity to Congo red by means of $Ba(OH)_2$ this salt was reprecipitated by dropwise addition to ethanol. The acid barium salt now separated in microcrystalline form and was analytically pure. On filtering off by suction, washing with alcohol, and drying under vacuum, a yield of 8.65 g. or 90% of the theoretical amount was obtained. The compound dried under high vacuum has the empirical formula $C_5H_9O_9PBa$, which corresponds to $PO_3Ba - O - CH_2 - (CHOH)_3 - COOH$. (The attachment of Ba to the OH group is arbitrary.)

Anal. Calcd.: C, 15.7; H, 2.4; P, 8.1; Ba, 36.0%. Found: C, 15.8; H, 2.7; P, 8.0; Ba, 36.2%.

To determine the optical rotation, 0.7369 g. of the acid barium salt was dissolved in 4 ml. 1 *N* HCl and the solution was made up to 10 ml. with water. This corresponds to 0.4750 g. of 5-phospho-D-arabonic acid in 10 ml. After 15 min. $[\alpha]_D^{24} = +10.8°$ ($\alpha = +1.03°$; $l = 2$; $c = 4.75$). After 36 hr. the rotation had changed to +17.9°. This change is due to lactone formation, not to hydrolysis, as 5-phospho-D-arabonic acid is extremely stable to both acid and alkaline hydrolysis.[3] In this respect it resembles its lower homolog 3-phosphoglyceric acid. Like the latter, too, it is easily cleaved by acid or alkaline phosphatase.

The starting material for the preparation of 5-phospho-D-arabonic acid, i.e., D-fructose 6-phosphate is easily prepared (4,5) or commercially available from Schwarz Laboratories.

Attempts to prepare phosphoarabonic acid by direct chemical degradation of fructose 1,6-diphosphate were unsuccessful, even when the now available soluble salts of the diphosphate (5) were used as the starting material. It is not necessary, however, to start with the isolated D-fructose 6-phosphate. The first product obtained on partial hydrolysis of the alkaline earth salts of fructose diphosphate with oxalic or sulfuric

[2] Alternatively the seven fold volume of methyl alcohol may be taken. The separation of the acid barium salt in an easily filtered form can be facilitated by the addition of a few drops of an alcoholic solution of barium bromide or barium perchlorate.

[3] The fact that phosphoric acid is not split off during the conversion of D-fructose 6-phosphate to 5-phospho-D-arabonic acid is due to the relatively great strength of the bond between the phosphoric acid residue and the primary alcoholic group in position 6 or 5.

acid may be utilized. After the acids used for hydrolysis are removed by precipitation with $Ba(OH)_2$, oxygen is bubbled through. After adjusting the pH with $Ba(OH)_2$, the resulting solution is treated exactly as described above. Because of admixtures still present it is, however, necessary to reprecipitate the acid barium 5-phospho-D-arabonate 4–5 times in this case. Even so, the yield is about 60–65% of the theory.

Discussion

Acids of the carbohydrate series as such are, as a rule, much more resistant than their phosphorylated derivatives toward biochemical transformations, resulting in a clear-cut controlled elimination of one terminal C-atom from the C-chain. Examples for this behavior can be found in the phosphorylated glyceric acids as compared to glyceric acid; only the former, the 3-phosphoglyceric acid as well as the 2,3-diphosphoglyceric acid, are fermented by yeast under cooperation of phosphatases and other enzymes to acetaldehyde and carbon dioxide (6). Similarly, phosphogluconic acid but not gluconic acid[4] may be biochemically converted to pentose derivatives (1). With the aid of phosphoarabonic acid various problems of carbohydrate metabolism may be studied.

Furthermore, the salts of this substance are some of those capable of solubilizing insoluble matter in cells, a phenomenon recently described by us (7). Because of its tendency to complex formation and its optical rotation, 5-phospho-D-arabonic acid appears of value for relevant investigations.

Summary

A simple method for the preparation of 5-phospho-D-arabonic acid by oxidation of D-fructose 6-phosphate is described. The significance of such an easily accessible phosphorylated product of the carbohydrate series obtainable in excellent yield is discussed.

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[4] The biological reaction is different from the oxidative decarboxylation of D-gluconic acid to D-arabinose by the degradation with H_2O_2 + Fe (Ruff) or by electrolysis or photocatalysis (Neuberg), cf. Ref. (8).

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