STUDIES ON D-TETROSE METABOLISM

I. PURIFICATION OF D-ERYTHROSE AND PREPARATION OF D-ERYTHRULOSE

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Despite a bulk of informations about the metabolisms of hexoses, pentoses and trioses have hitherto been obtained, relatively little attention has been given to the metabolisms of tetroses. One of the chief sources of difficulty in the biochemical study of D-tetrose lies in the preparation of the sugar. Several methods have been described for the preparation of D-erythrose. These have been surveyed by Overend *et al.* (1) and more recently briefly reviewed by Perlin and Brice (2, 3). All the procedures involve the degradation of an appropriate sugar derivative. The product contains usually impurities because tetroses have not yet been obtained in crystalline forms. A technique for the separation of sugars by Dowex 1 borate form column has been introduced by Khym and Zill (4—6). This procedure enables the separation of hexoses and pentoses (4—8). It was considered that the technique might equally well be applied to the purification of tetrose. In this paper a chromatographic method using Dowex 1 column for the purification of D-erythrose is described.

Among tetroses, D-erythrulose is the most poorly characterized sugar. Only one procedure for the formation of D-erythrulose has been reported by Iwadare (9), which involves the preparation of isopropylidene-D-glyceroyl chloride from D-mannitol *via* isopropylidene-D-glyceraldehyde, condensation of the acid chloride with diazomethane and hydrolysis of the resulting diazoketone. D-Erythrulose was prepared according to the method referred to above, but the final product was obtained only in a low yield and gave three or four spots on paper chromatograms. D-Ribulose (10), D-xylulose (11) and dihydroxyacetone (12) have been obtained from the corresponding aldoses by epimerization in hot pyridine. Iwadare (9) had reported that D-erythrulose failed to be obtained by heating D-threose with pyridine. Now, however, we have found that D-erythrulose can be obtained by heating D-erythrose with pyridine. It may be possible to obtain D-erythrulose from D-threose by a similar procedure. In the present paper a method for the preparation of D-erythrulose from D-erythrose is described, together with its characterization.

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EXPERIMENTAL AND RESULTS

1. Purification of D-Erythrose

D-Erythrose was prepared from calcium D-arabonate by Ruff's method (13), and ionic impurities were removed by treatment with Amberlite IR 120 (H^+ form) resin and Duolite A 4 (OH^- form) resin according to the method described by Fletcher *et al.* (14).

The purification of D-erythrose was performed with 50–100 mesh Dowex 1 $\times$ 1 resin. The resin was washed with 2 *N* hydrochloric acid, converted to borate form by washing with 0.1 *M* sodium borate, followed by washing with water to remove the excess borate. The resin was then poured into a column and washed with 0.005 *M* sodium borate prior to passing through the sugar solution. 60 mg of D-erythrose crude syrup was dissolved in 30 ml of 0.005 *M* sodium borate, adsorbed on a 0.84 $cm^2 \times 66.5$ cm column, and eluted with 0.005 *M* sodium borate. The flow rate was 0.6 ml per minute. The eluate fractions containing D-erythrose were conveniently detected by silver-mirror test and D-erythrose content was determined by the method of Dische (15). Fig. 1 illustrates a typical result. To obtain D-erythrose from the appropriate eluate fractions, the fractions were passed through a column of Dowex 50 (H^+ form) to convert the borate to the free acid and the solutions were evaporated to dryness *in vacuo* at 30°. Absolute methanol was then added and the methyl borate was removed by distillation (6). This process was repeated three times and the final syrup was dissolved in water and any insoluble residue was removed by filtration.

FIG. 1 Purification of D-Erythrose

A solution of 60 mg of crude D-erythrose in 30 ml of 0.005 *M* sodium borate was adsorbed on a 0.84 $cm^2 \times 66.5$ cm column of Dowex 1 $\times$ 1 borate form resin, 50 to 100 mesh, and eluted with 0.005 *M* sodium borate. The flow rate was 0.6 ml per minute.

Separation of D-erythrose from impurities was satisfactory. On paper chromatograms, D-erythrose obtained from the Dowex 1 column gave a single spot, in contrast with the crude D-erythrose showing at least three spots. The paper chromatograms were developed by an ascending technique on Toyo filter paper No. 50, 2 $\times$ 40 cm, in iso-propanol-water (6:1). Silver nitrate (16) and aniline phthalate (17) were used as

the locating reagents. Thus about 30 mg of chromatographically pure D-erythrose was obtained from 60 mg of crude D-erythrose under the above conditions.

2. Preparation of D-Erythrulose

Treatment of D-Erythrose with Pyridine—In a 200-ml round-bottomed flask, 10 g of D-erythrose and 100 ml of dry pyridine were refluxed for 4 hours, on an oil bath at the bath temperature of about 130°, with a condenser having a calcium chloride tube to exclude moisture. The brown colored solution was evaporated under reduced pressure to remove pyridine, and the residual syrup was dissolved several times in methanol and reevaporated as thoroughly as possible at the bath temperature of 40–45°. The brown syrup was then dissolved in 50 ml of water and any insoluble residue was removed by filtration. The solution was concentrated to a syrup and dried *in vacuo*.

On paper chromatograms D-erythrose has an R_F of 0.65 when the solvent is phenol-water (3:1). By treating D-erythrose with pyridine two small spots appeared on paper chromatograms, one corresponding to D-threose (R_F 0.58) and the other being an unknown compound (R_F 0.74). D-Threose was prepared from D-galactose according to the method of Perlin and Brice (18). When D-threose was similarly treated with pyridine, formation of a considerable amount of D-erythrose and a small amount of an unknown compound having the same R_F as the compound produced from D-erythrose was observed. It was therefore, as expected, apparent that epimerization of D-erythrose and D-threose has been brought about by pyridine treatment. The unknown product having an R_F of 0.74 seemed to be D-erythrulose because in the epimerization the two epimeric aldoses and the corresponding ketose are expected to be in equilibrium.

Separation of D-Erythrulose by Dowex 1 Column—Borate form of 50–100 mesh Dowex 1×1 resin was poured into a column and washed with 0.05 M boric acid prior to passing through the sugar. 1.3 g of D-erythrose treated with pyridine was dissolved in 100 ml of 0.05 M boric acid, adsorbed on a 1.45 cm²×38 cm column, and eluted with 0.05 M boric acid. The flow rate was 1.5 ml per minute, and 30 ml of the samples were collected. The first fractions giving a positive silver-mirror test were eluted in 50 to 400 ml of the eluates. These fractions were discarded. The second fractions were eluted in 500 to 1800 ml, in which D-erythrulose was contained. The second fractions were collected and concentrated under reduced pressure to dryness. The residue was treated three times with methanol and finally the pale yellow syrup was dissolved in water and any insoluble material was removed by filtration. About 70 mg of the product was obtained, which gave a single spot on paper chromatograms, from 1.3 g of D-erythrose treated with pyridine.

Characterization of D-Erythrulose—155 mg of the compound obtained in this way was dissolved in 1 ml of methanol. A solution of 198 mg of *o*-nitrophenylhydrazine in 5 ml of methanol and a few drops of glacial acetic acid were added.

The mixture was warmed on a bath for 10 minutes at the bath temperature of 60–65°, and stored in the dark overnight. The solution was then evaporated under reduced pressure. The red oily residue was dissolved in water at 60–70° and any insoluble residue was removed by filtration. The crystalline product was obtained upon cooling as orange needles. Recrystallized five times from water, mp 116–117°. Calculated for $C_{10}H_{13}O_5N_3$: C, 47.06; H, 5.15; found: C, 47.45, 47.66; H, 4.97, 4.96 %.

Thus the compound obtained from D-erythrose by treatment with pyridine is concluded to be D-erythrulose.

Paper Chromatography of D-Tetroses—D-Erythrose, D-threose and D-erythrulose obtained in this way were subjected to paper chromatography with a number of solvents (Table I).

TABLE I
R_F Values of D-Tetroses in Various Solvents

Chromatograms were developed by the ascending technique on Toyo filter paper No. 50, 2×40 cm. The sugars were located by spraying the papers with aniline phthalate, benzidine or naphthoresorcinol reagent.

Solvent system	<i>R_F</i> values		
	D-Erythrose	D-Threose	D-Erythrulose
Phenol-water (3:1)	0.65	0.58	0.74
<i>n</i> -Butanol-half saturated with water	0.23	0.30	0.30
<i>n</i> -Butanol-ethanol-water (4:1:5)	0.34	0.40	0.40
<i>iso</i> -Propanol-water (6:1)	0.52	0.58	0.58

In most cases, except in phenol-water, D-erythrulose failed to be differentiated from D-threose by difference in *R_F* value. However, D-erythrulose gave a different color from D-aldotetroses on spraying with aniline phthalate (17), benzidine (19), or naphthoresorcinol (19) reagent. D-Erythrose and D-threose gave dark yellow color with aniline phthalate, brown with benzidine, and blue spot, having an intense blue fluorescence in ultraviolet light with naphthoresorcinol, whereas D-erythrulose gave yellow color with aniline phthalate, orange red with benzidine, and no color with naphthoresorcinol. These differences in colors readily distinguished D-erythrulose from aldotetroses.

When aniline phthalate reagent was prepared with acetic acid instead of water-saturated butanol, tetroses were visualized more distinctly.

DISCUSSION

Dische and Dische (15) have reported two specific color reactions of tetroses. Contrary to the experience of Dische, we observed that D-erythrulose obtained from D-erythrose by epimerization gave much less intense colors than that produced by D-erythrose in Dische reactions. The source of this variance remains to be investigated.

SUMMARY

1. A method for the purification of D-erythrose using Dowex 1 borate column has

been presented.

2. A product formed from D-erythrose by treatment with pyridine has been isolated by Dowex 1 borate column chromatography and characterized as D-erythrulose by preparation of the crystalline *o*-nitrophenylhydrazone.

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