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PREPARATION OF D-RIBOSE FROM D-ERYTHROSE BY CYANOHYDRIN REACTION

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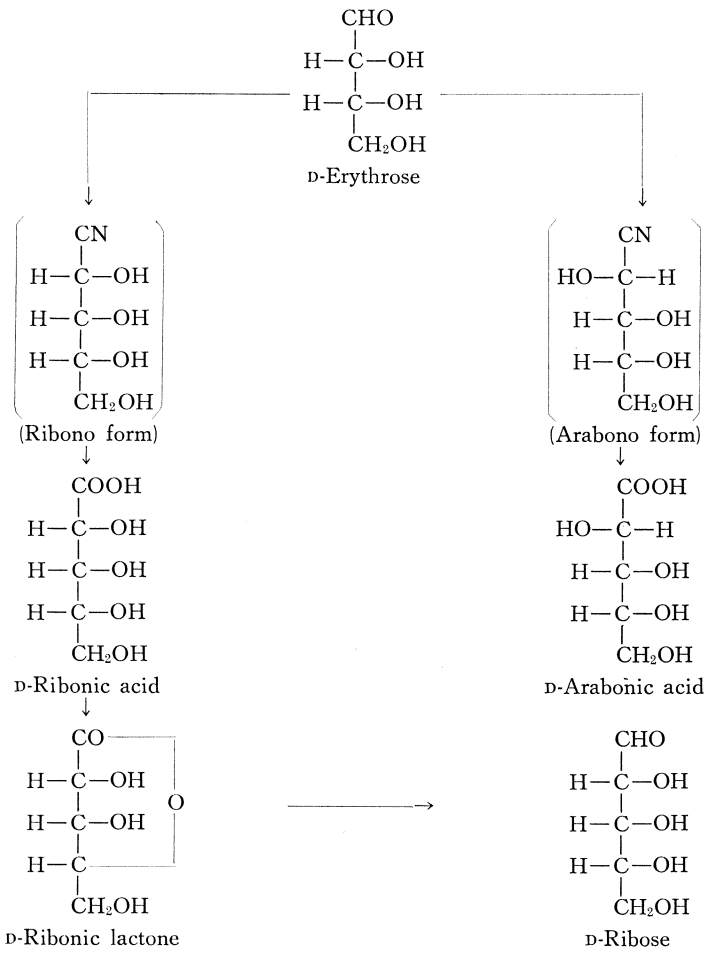
The preparation of the next higher aldoses by the cyanohydrin synthesis has been reported by many workers (1), but D-ribose has not been synthesized from D-erythrose. We have undertaken the application of the cyanohydrin synthesis to D-



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erythrose. D-Ribose has been synthesized by the addition of hydrogen cyanide to D-erythrose, followed by fractional crystallizations of the sugar acid salts, and subsequent hydrogenation of the sugar acid lactone according the route given in the diagram.

EXPERIMENTAL

Materials and Methods

D-Erythrose was prepared according to the procedure of Perlin and Brice (2), and estimated colorimetrically by Dische's method (3), and volumetrically by iodometry (4).

Lactone was determined according to Lipmann and Tuttle (5). Pentose was estimated colorimetrically by Bial's orcinol method (6).

Reducing sugar content during reduction of lactone was estimated by iodometry (4) and color reaction with aniline and hydrochloric acid. (7).

Preparation of D-*Ribose*

1. D-*Ribonic Acid from* D-*Erythrose*

D-Ribonic acid was prepared from D-erythrose as described for the preparation of D-allonic acid by Levene *et al.* (8).

A solution of 20 g (0.167 mole) of D-erythrose in 127 ml of water was cooled to 0° and to this, 7.3 ml (0.188 mole) of hydrogen cyanide was added. After adding a few drops of aqueous ammonium, the reaction mixture was allowed to stand for 24 hours at 14°. At this time, the solution did not show any reducing power. The dark brown solution was diluted with 500 ml of water and to this was added 37.5 g of barium hydroxide eighthydrate. The reaction mixture was heated on a water bath until ammonia was expelled completely. The barium ions were removed by the addition of an equivalent amount of sulfuric acid. To the filtrate was added calcium carbonate until the solution was neutral. After centrifugation, the precipitate was discarded. The supernatant was concentrated under reduced pressure. Upon standing in the refrigator, calcium D-arabonate hydrate was crystallized. Concentration of mother liquors yielded further amounts; yield 16 g. The filtrate was diluted with 200 ml of water and the solution was passed through a column of Amberlite IR-120 (H^+) to convert the calcium salt of D-ribonic acid to the free acid. The column effluent was concentrated under reduced pressure to a thick sirup (10.0 g).

2. Preparation of D-*Ribonic Lactone from* D-*Ribonic Acid*

D-Ribonic lactone was prepared from D-ribonic acid as described for the preparation of D-aldonic lactone by Fischer *et al.* (9), and Isbell (10).

After adding 200 ml of ethyl acetate to 10 g sirupy D-ribonic acid, the mixture was heated for two to three hours with reflux on a water bath. It was concentrated to a thin sirup and the brown residue was dissolved in 50 ml of water. After decolorizing with charcoal, the solution was concentrated to a thin sirup in reduced pressure. The residue was heated with 50 ml of *n*-butanol on an oil bath at 140° and *n*-butanol was removed from the mixture by distillation. The residual sirup was

dissolved in 50 ml of water and after decolorizing with charcoal, the solution was concentrated to a thin sirup (8.7 g).

The paper chromatogram of sirupy D-ribonic lactone thus obtained gave two spots. One of them showed the same R_f value as D-arabonic lactone (Fig. 1), and another one showed a positive lactone test and gave D-ribose by reduction. The result of paper chromatography revealed that D-ribonic lactone was moved faster than D-arabonic lactone.

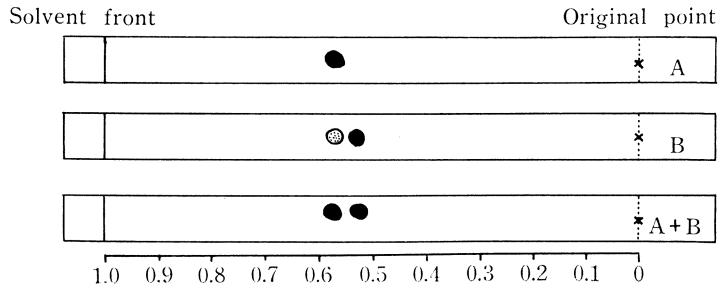

FIG. 1 *Paper Chromatograms of* D-*Ribonic Lactone*
Chromatograms were developed by an ascending technique on Toyo filter paper No. 50, 2 × 40 cm, in isopropanol-water (6 : 1) for 16 hr at 18°. The lactones were located by spraying the papers with hydroxylamine-$FeCl_3$ reagent and silver nitrate reagent.
A, D-arabonic lactone; B, D-ribonic lactone prepared.

3. *Reduction of* D-*Ribonic Lactone*

D-Ribonic lactone was reduced with sodium amalgam as described for the reduction of D-allonic lactone by Pratt *et al.* (11). A solution of 5.5 g of the lactone in 660 ml of oxalate-oxalic acid buffer (pH 3.0), containing 6.8 g of oxalic acid dihydrate and 13.6 g of sodium oxalate, was chilled to about 0°. During the reaction, the pH of the solution was maintained by adding oxalic acid immediately before the addition of each 50 g portion of 2% sodium amalgam. The alternate addition of oxalic acid and sodium amalgam was continued until no further increase in reducing sugar. The reaction mixture was cooled to 0° and agitated vigorously. The reaction was stopped and the aqueous phase was decanted from the mercury. The oxalate ions were removed by the addition of an equivalent amount of 20% calcium chloride. The filtrate was concentrated under reduced pressure to a small volume. The crystallized salt was filtered and the filtrate was diluted with about 100 ml of water. The solution was passed through a column of Amberlite IR-120 (H^+) to remove the cation and the column effluent was neutralized by addition of Amberlite IR-45 (OH^-). The resin was filtered off and washed. After treatment with charcoal, the filtrate and washings were concentrated under reduced pressure to a thin sirup (6.3 g). From the above synthetic mixture, D-ribose was separated by partition chromatography on a column of powdered cellulose.

4. *Purification of* D-*Ribose*

Cellulose column chromatography was carried out according to the procedure

of Hough *et al.* (12). Cellulose powder (Toyo Roshi A, 100-200 mesh) was suspended in the eluent, *n*-butanol half saturated with water, and the whole was introduced in a 3.5 cm diameter column to a height of 43 cm. A sirup of 2 g of crude D-ribose was dissolved in the minimum quantity of water, and the solution was applied to the above column. Sugars and lactones were eluted with use of *n*-butanol half saturated with water. The effluent was collected in 15 ml fractions at 1 ml per minute, with the aid of an automatic fraction collector. The progress of a chromatographic separation on the cellulose column was followed by appropriate color reactions for the detection of sugars and lactones. The lactones were found in the first fraction (140 ml) of the eluate, D-ribose in the second fraction (170 ml), and D-arabinose in the last fraction (280 ml), as shown in Fig. 2. D-Ribose fraction contained no other sugars and gave a pure crystalline product, mp 86°, on crystallization from ethanol.

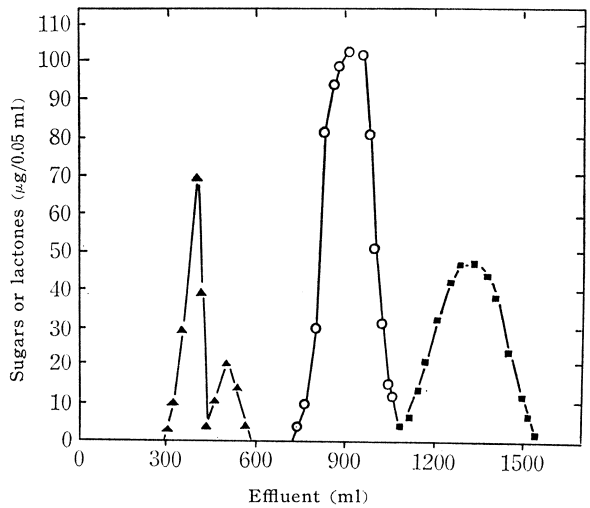

FIG. 2 *Chromatography of Crude* D-*Ribose on Cellulose Column*
A solution of 2.0 g of crude D-ribose in 1.0 ml of water was adsorbed on a 6.97 cm^2 × 43 cm column of cellulose powder, 100 mesh, and eluted with *n*-butanol, half saturated with water. The flow rate was 1 ml per minute. ○, D-ribose; ■, D-arabinose; ▲, lactones.

RESULTS AND DISCUSSION

Hudson (13) has reported that, when hexonic acid are synthesized from L-arabinose by cyanohydrin synthesis, if cyanide addition is carried out in a cold aqueous solution of sodium cyanide, L-gluconic acid is the principal product with only a minor amount of L-mannoic acid, whereas the use of aqueous hydrocyanic acid give mainly the products with L-mannoic acid.

When sodium cyanide was used for the preparation of the cyanohydrin of D-erythrose, reducing power of the reaction mixture was not disappeared even after a few days, probably because of insufficient progress of the cyanide addition. On

the other hand, reducing power was almost disapeared after a day, when aqueous hydrocyanic acid was used in the presence of a little ammonia. After hydrolysis of the cyanohydrin of D-erythrose, D-arabonic acid was crystallized as calcium salts in a yield of 26 %.

After the removal of calcium ions, lactonation of the filtrate afforded the desired lactones in a yield of 22 %, although paper chromatograms of the products showed that the lactones fairly contained D-arabonic lactone. Reduction of the crude lactones by sodium amalgam affoaded the mixture of D-ribose and D-arabinose in a yield of 58 %.

Chromatographic separation of the reduction products on a cellulose column revealed that the content of D-ribose in the mixture was 42 %. Accordingly, the over-all yield of D-ribose from D-erythrose was about 5 %. From the results of the preparation of D-ribose, it is evident that the asymetric synthesis has favored the formation of *trans*-hydroxyl groups for the new asymetric center.

SUMMARY

1. The synthesis of D-ribose from D-erythrose by cyanohydrin reaction was carried out.

2. The product formed in largest quantity from D-erythrose has an arabonic configuration under the original conditions of Kiliani and Fischer (1).

3. The result of paper chromatography with use of a solvent system, isopropanol-water (6 : 1) revealed that D-ribonic lactone moved faster than D-arabonic lactone.

4. The crude D-ribose obtained by reduction of D-ribonic lactone was purified with use of cellulose column chromatography according to Hough *et al.* (12).

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REFERENCES

1. Sowden, J. C., The Carbohydrates, p. 106, edited by W. Pigman, Academic Press, New York, 1957.
2. Perlin, A. S. and Brice, C., *Can. J. Chem.*, **33,** 1216 (1955).
3. Dische, Z. and Dische, M. R., *Biochem. Biophys. Acta.*, **27,** 184 (1958).
4. Willstätter, R. and Schudel, G., *Ber. duet. chem. Ges.*, **51,** 780 (1918).
5. Lipmann, F. and Tuttle, L. C., *J. Biol. Chem.*, **159,** 21 (1945).
6. Bial, M., *Biochem, Z.*, **3,** 323 (1906).
7. Koch, F. C. and Hanke, M. E., Practical Methods in Biochemistry, p. 17 (1948).
8. Levene, P. A. and Jacobs, W. A., *Ber. duet. chem. Ges.*, **43,** 3141 (1919).
9. Fischer, E. and Piloty, O., *Ber. duet. chem. Ges.*, **24,** 4212 (1891).
10. Isbell, H. S. and Frush, H. I., *Bureau. Stand. J. Research*, **11,** 662 (1933).
11. Pratt, J. W. and Richtmeyer, K. N., *J. Am. Chem. Soc.*, **77,** 1906 (1955).
12. Hough, L., Jones, J. K. and Wadman, W. H., *J. Chem. Soc.*, **1949,** 2511 (1949).
13. Hudson, C. S., *J. Am. Chem. Soc.*, **73,** 4498 (1951).