PREPARATION OF 1-C^{14} PENTONIC ACIDS BY THE CYANHYDRIN SYNTHESIS[1]

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ABSTRACT

Solutions of pure D-threose and D-erythrose, buffered with bicarbonate (0.82 moles) plus carbonate (0.18 moles), were treated with an equimolar amount of C^{14}-labeled KCN. The aldonic acids were isolated as salts, using carrier technique, in yields of 90–95% based on the radioactive carbon. D-Erythrose gave 1.89 times as much arabonic as ribonic acid while D-threose gave 2.36 times as much lyxonic as xylonic acid. Despite the unfavorable ratio in the latter case it was found that D-xylose could be obtained in an over-all yield of 22%, based on the cyanide.

Interest in the metabolism of pentoses by bacteria has prompted an investigation on the cyanhydrin synthesis as a method for preparing 1-C^{14}-labeled pentoses. L-Arabinose-1-C^{14} has been made, in yields of about 3% (12), by the nitromethane synthesis while D-xylose-1-C^{14} (13) has been prepared from glucose-1-C^{14}. As far as the present writer knows pentonic acids have never been prepared by the cyanhydrin reaction, so there is no information on the yields of isomers to be expected. The ratio of epimers would be expected to vary with the pH of the cyanide reaction mixture (10) and a complete investigation should include a study of this important factor. The present paper gives information on the yields of pentonic acids obtained from D-erythrose and D-threose under one set of conditions. Since the results indicate that the cyanhydrin reaction is a convenient route for preparing C^{14}-labeled pentoses this information may be of value to other workers.

Deionized solutions of threose or erythrose, prepared by the Ruff degradation, contained at least one other reducing substance so they were not used. D-Erythrose was obtained from 4,6-ethylidene-D-glucitol (9), the intermediate 2,4-ethylidene-D-erythrose being purified by distillation. D-Threose was prepared from 1,3-benzylidene-D-arabitol. The intermediate 2,4-benzylidene-D-threose crystallized, unexpectedly, so it was not necessary to convert it to the isopropylidene derivative for purification. The solutions of tetroses obtained by these methods were pure as far as could be ascertained by paper chromatography.

The cyanhydrin reaction and hydrolysis of the amides was done following the work of Isbell *et al.* (10) on preparation of D-glucose-1-C^{14}. The aldonic acids were separated using methods developed for epimerization studies (3, 4). Since high specific activities were not required, these separations were simplified by the use of carriers. The yields were good, 90–95% of the C^{14} being recovered as aldonic acids. D-Erythrose gave arabonic and ribonic acids in the ratio of 1.89: 1 while D-threose gave lyxonic and xylonic acids in the ratio of 2.36: 1 respectively.

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The conversion of these acids to the aldoses has not been thoroughly investigated. It has been pointed out by Isbell *et al.* (10) that there is no universal procedure for the sodium amalgam reduction of lactones which will give maximum yields with all lactones. They found that glucono-δ-lactone gave excellent yields in an oxalate buffered medium while mannonic-γ-lactone gave better yields in a benzoate buffered medium and required more amalgam. We have investigated the reduction of xylono lactone and obtained better yields in an oxalate buffered medium than in a benzoate buffered one but it required twice as much amalgam as glucono-δ-lactone. The yield of xylose was about 80%, based on the cadmium D-xylonate cadmium bromide double salt. Presumably the other aldonic acids could be converted to pentoses in similar yields under the best conditions. D-Arabinose-1-C^{14} could then be used for preparation of D-glucose-2-C^{14} in good yields, by addition of inactive cyanide (10). In both of the cyanhydrin reactions, starting with D-erythrose, the epimer needed for this synthesis is formed in the highest yield.

EXPERIMENTAL

Methods

Unless stated otherwise evaporations were carried out in a Craig evaporator (2) using a bath temperature of 40–45° C. and the full vacuum of a filter pump (20–25 mm.). The aldose content of the solutions was determined by hypoiodite oxidation (8). Measurements of radioactivity were made using a windowless counter operating in the proportional region. The samples were plated on aluminum disks and were so small that self absorption was negligible. The counting assembly was calibrated against the U.S. National Bureau of Standards carbon-14 standard; a sodium carbonate solution rated at 1280 disintegrations per second/ml. The results were expressed as millicuries. Paper chromatography of the tetrose solutions was carried out with Whatman No. 1 paper using the descending technique. Methyl ethyl ketone saturated with water was the only developing solvent used. Spots of reducing substances were detected by three spray reagents; molybdate (1), alkaline dinitrosalicylate (11) and triphenyl-tetrazolium chloride (15). The tetrazolium spray is much more sensitive for tetroses than glucose, whereas the other sprays are about equally sensitive for glucose and the tetroses.

Preparation of Tetroses by the Ruff Degradation

Ruff degradation (5) of Ca-D-arabonate and Sr-D-xylonate gave solutions of D-erythrose and D-threose. Analysis of the deionized solutions for aldose indicated yields of 36% and 40% of the theoretical, respectively. However, paper chromatography showed at least two reducing substances to be present in each solution, both being detected by all three sprays as spots of about equal intensity. One component migrated at the same rate as the tetroses, and the other at about half the rate. A pure tetrose fraction could probably be obtained by chromatographic fractionation of these solutions. However the tetrose solutions used in the cyanhydrin reactions were prepared by other methods, described below, which gave chromatographically pure solutions directly.

2,4-Ethylidene-D-Erythrose

4,6-Ethylidene-D-glucitol (10.4 gm., 50 mM.) (9) was dissolved in 150 ml. of ice-cold water and treated with an ice-cold solution containing 23.94 gm. (105 mM.) of H_5IO_6 in 150 ml. of water. After 10 min. the mixture was neutralized to the phenolphthalein end point by a warm concentrated solution of strontium hydroxide. The precipitate was filtered out and the filtrate evaporated to dryness. The residue was extracted with alcohol, the alcohol evaporated, and the sirup distilled through a short column, under reduced pressure, to give 1.5 gm. of 2,4-ethylidene-D-erythrose, b.p. 98° C. at 8 mm., as the only fraction. This material was a colorless glass; $[\alpha]_D^{22}$ $-41.36°$ (c, 2.5, water). It gave 91.2% of the expected amount of acetaldehyde on hydrolysis by 0.5 N sulphuric acid, followed by distillation, as measured by the bisulphite binding capacity of the distillate. This rotation is a somewhat higher than the value $[\alpha]_D$ 36, reported for the L-isomer (12).

D-*Erythrose*

A solution of 1.08 gm. (7.4 mM.) of 2,4-ethylidene-D-erythrose was dissolved in 45 ml. of water and 6–8 ml. of IR-120-H Amberlite cation-exchange resin added. This mixture was distilled at constant volume for 1.5 hr. to effect hydrolysis and removal of the acetaldehyde. The residue was treated with charcoal and filtered. The colorless solution thus obtained was found to contain 5.71 mM. of aldose. A portion was chromatographed on paper as described above and found to give only one spot with an R_f of 0.21 (glucose had R_f of 0.055 on same sheet). An aliquot of this solution was used for the cyanhydrin synthesis described below.

Reaction of C^{14}-labeled Cyanide with D-*Erythrose*

A solution containing 2 mM. of $KC^{14}N$ (1 millicurie) and 0.36 mM. of KOH in 10 ml. of water was frozen solid in a 50 ml. glass stoppered Erlenmeyer flask immersed in a dry ice bath. An aliquot of the D-erythrose solution (14.1 ml., 2 mM.) containing 2 mM. of $NaHCO_3$ was added, the stoppered flask allowed to stand two days at room temperature, and then 1.64 ml. of N NaOH added. After two more days at room temperature the mixture was heated to 60° C. and aerated to remove the ammonia released by hydrolysis of the amides. This ammonia was caught in standard acid and measured by back titration. A total of 1.85 m.e. (92.5%) was recovered in five hours, at which time the volume had decreased to about one-third and the hydrolysis was complete. The residue was transferred to a beaker containing 25 ml. of IR-120-H Amberlite cation-exchange resin, stirred, and then transferred to a column containing an additional 25 ml. of the resin. The effluent was treated with 2 gm. of Ca-D-arabonate carrier and the mixture warmed with 0.3 gm of calcium carbonate, filtered, and the filtrate concentrated to about 8 ml and crystallization induced by addition of methanol. The Ca-D-arabonate which separated (2.3 gm., 0.635 mc.) was recrystallized from methanol–water to constant specific activity to give 2.25 gm. (0.590 mc.). The mother liquors were combined, concentrated to remove methanol, diluted with water, passed through an IR-120-H column to remove the calcium ions, boiled with 0.4 gm of cadmium carbonate in the presence of 2 gm. of Cd-D-ribonate carrier, filtered, and the filtrate concentrated and crystallized from methanol–water. After recrystallizing to constant specific activity, 1.97 gm. (0.312 mc.) of Cd-D-ribonate was obtained. Thus the ratio of arabonate: ribonate was 1.89, and a total of 90.2% of the C^{14} added as cyanide was found in the aldonic acids.

*2,4-Benzylidene-*D*-Threose*

A solution of 5 gm. of 1,3-benzylidene-D-arabitol (7) in 150 ml. of warm water was cooled to 30° C. and mixed with 50 ml. of 9.45% $NaIO_4$. After one hour at room temperature, 14.5 ml. of molar barium acetate was added. The mixture was filtered and the filtrate concentrated in a flask containing 1 gm. of $BaCO_3$. The residue was dehydrated by three successive evaporations with 25 ml. portions of absolute ethanol, then dissolved in ethanol and filtered. The filtrate was concentrated to a sirup and allowed to stand one hour at room temperature. This was treated with 100 ml. of water, and a crystalline residue formed, which was filtered out and washed with water giving 2.4 gm. melting at 160–161° C. This melting point was raised to 165.5° C. by recrystallization from ethanol. Found: $[\alpha]_D^{22}$ 77.6° (*c*, 2, pyridine), C, 63.32; H, 5.82. Calculated for $C_{11}H_{12}O_4$: C, 63.45, H, 5.81. This compound was obtained only as a sirup by previous workers (14), who carried out this oxidation using lead tetraacetate. However, a crystalline benzylidene-L-threose hemihydrate, m.p. 119–120° C., has been reported (6).

D-*Threose*

A solution of 1.7 gm. of 2,4-benzylidene-D-threose in 40 ml. of 10% acetic acid was refluxed for one hour, concentrated to a sirup, and the residue, after addition of 20 ml. of water, was extracted three times with ether to remove the last traces of benzaldehyde. The aqueous phase was again evaporated to a sirup and dissolved in 20 ml. of water to give a clear colorless solution, pH 5.0, containing 7.22 millimoles (88%) of aldose. Paper chromatography as described above showed the presence of only one reducing substance with an R_f of 0.22 (glucose had R_f of 0.055 on the same sheet). A portion of this solution was used in the cyanhydrin synthesis described below.

Reaction of C^{14}-labeled Cyanide with D-*Threose*

This reaction was carried out with 2 mM. (1 mc.) of cyanide as described for D-erythrose. The yield of ammonia on hydrolysis was 1.8 m.e. (90%). The solution of aldonic acids obtained, after removal of the cations, was warmed with 2 mM. of cadmium hydroxide and 5 mM. of cadmium carbonate, then treated with 10 m.e. of aqueous HBr and filtered. Inactive cadmium-D-xylonate cadmium bromide double salt (2 gm.) was dissolved in the filtrate, which was then concentrated until crystallization started. After this was allowed to stand overnight at 3° C., 1.98 gm. of the double salt was obtained. Recrystallization to constant specific activity from water, and reworking of the mother liquors, gave a total of 2.17 gm. (0.273 mc.) of cadmium xylonate cadmium bromide. Addition of more carrier to the combined mother liquors, followed by reisolation, gave only 0.011 mc. more. Thus a total of 28.4% of the C^{14} was present as D-xylonic acid.

The combined mother liquors from the double salt were passed through an IR-120-H column containing 50 ml. of resin. The effluent was stirred one hour with 4 gm. of silver carbonate, filtered, and the bromide-free filtrate treated with 2 gm. of D-lyxono lactone and passed through a 25 ml. column of IR-120-H resin, to remove the silver ions. The effluent was concentrated to a sirup which was dehydrated by three evaporations with glacial acetic acid, giving finally 3 gm. of sirup. This was dissolved in 2 ml. of glacial acetic acid and cooled. Crystallization of the lactone was rapid. The product was recrystallized twice from acetic acid – ethyl acetate (about equal proportions) to give 1.73 gm. (0.260 mc.) of D-lyxono lactone. The mother liquors still contained a considerable amount of C^{14}, possibly because equilibrium had not been reached between the carrier lactone and the active lyxonic acid. In order to confirm this supposition another 2 gm. of inactive D-lyxono lactone was added to the mother liquors, the acetic acid removed by evaporation with water, and the aqueous solution titrated with N KOH to the phenol red end point until all the lactone was converted to the K salt. The potassium ions were then removed by an IR-120-H column and the effluent concentrated to a sirup and crystallized from glacial acetic acid as before. This time the crystallization required four days. After purification to constant specific activity 1.1 gm. (0.210 mc.) of D-lyxono lactone was obtained. The residue from this was rather gummy so another gram of carrier was added and reisolated by rapid crystallization giving another 1.1 gm. (0.100 mc.) of pure D-lyxono lactone. A total of 0.570 mc. of pure lactone was isolated and it was calculated, from the amounts of carrier added and recovered, that another 0.1 mc. was present in the residue. From these data about 67% of the C^{14} added as cyanide was converted to D-lyxonic acid. The ratio of lyxonic: xylonic acids was thus 2.36 and about 95% of the cyanide was converted to aldonic acids.

D-*Xylose-1-*C^{14}

Cadmium D-xylonate cadmium bromide (2.17 gm., 0.273 mc.) was dissolved in 125 ml. of warm water and the solution passed through a 50 ml. column of IR-120-H resin. The effluent was treated with 3.5 gm. of silver carbonate, filtered, and the bromide-free filtrate passed through a 35 ml. column of IR-120-H resin to remove silver ions. The effluent was evaporated to a sirup which was heated in a drying pistol, containing phosphorus pentoxide, at 100° C. (2 mm.) for 24 hr. to effect lactonization. The mixture of sirup and crystals thus obtained was dissolved in 120 ml. of water containing 4.2 gm. of oxalic acid dihydrate and 4.8 gm. of sodium oxalate. The mixture was stirred vigorously and 14 gm. of 5% sodium amalgam added. The temperature was maintained at 5–10° C. (ice-alcohol bath) and powdered oxalic acid added from time to time to keep the pH from rising above 4. After 30 min. another 14 gm. portion of the amalgam was added. When this was used up (about 45 min.) the mixture was filtered to remove mercury and excess sodium acid oxalate, the filter cake being washed with ice water. The filtrate was concentrated to about 50 ml., treated with 100 ml. of methanol, filtered again, diluted with an equal volume of water and neutralized by N NaOH, to the phenol red end point, to convert any residual lactone to the sodium salt. Ordinary D-xylose (3 gm.) was dissolved in the solution which was then deionized by 50 ml. columns of IR-120 and IR-4B Amberlite resins in the acid and base forms, respectively. The deionized solution was concentrated to a sirup which was dehydrated by three evaporations with absolute ethanol and then crystallized from ethanol containing a little isopropanol. The first crop of crystalline D-xylose was 3.17 gm. (0.194 mc.). It was recrystallized by solution in 1 ml. of hot water followed by addition of 2 ml. of methanol and 4 ml. of ethanol. This gave 2.97 gm. with unchanged specific activity. A further 4 gm. of carrier was added to the mother liquors and another 3.52 gm. (0.027 mc.) of pure D-xylose isolated. The pure xylose isolated contained about 77% of the activity present in the cadmium double salt, another 3–4% remaining in the mother liquors.

Some glyoxylic acid, isolated as the 2,4-dinitrophenyl-hydrazone, was formed from the oxalate during reduction by sodium amalgam. It was necessary to remove this aldehyde-acid by deionization of the solution before the yield of aldose could be determined by the hypoiodite method. Experiments on the reduction of a sample of crystalline D-xylono lactone using acetic acid or benzoic acid as buffers gave only 35–40% yields of aldose. These yields were not increased by addition of an extra portion of sodium amalgam.

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