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The Synthesis of D-Arabinose-1-^{14}C and D-Ribose-1-^{14}C

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A method for the synthesis of D-ribose-1-^{14}C and D-arabinose-1-^{14}C is described.

For certain biochemical investigations, we required some of the aldopentoses labelled with ^{14}C in the $C_{(1)}$ position, particularly D-ribose-1-^{14}C. To prepare these sugars we decided upon the cyanohydrin method, using D-erythrose as the starting material. When this work was undertaken Isbell *et al.*[1] had published methods for the synthesis of D-glucose-1-^{14}C and D-mannose-1-^{14}C from D-arabinose by the above method. Later when the present work was approaching completion they reported the synthesis of D-ribose-1-^{14}C and D-arabinose-1-^{14}C from D-erythrose [2]. Experimental details of their work has not yet been available to us.

The cyanohydrin synthesis of aldoses involves the addition of KCN to the one lower member of the homologous sugar series. The mixture of the two epimeric aldonic acids produced by hydrolysis of the nitriles is usually separated before the reduction of the lactones of the aldonic acids to the aldoses. Isbell *et al.*[1] separated the mixture of the D-gluconic and D-mannonic acids by fractional crystallization using the lactones and the barium salts. In the present case the reduction has been carried out with the mixture of the two epimers, and D-ribose and D-arabinose were finally separated by paper chromatography. For practical reasons, it was necessary to carry out the synthesis on a sufficiently small scale to enable the separation of the sugars on a few sheets of filter paper. For the sake of convenience no purification of the intermediates was attempted, although this resulted in a lower over all yield. Approx. 0.3 millimole K^{14}CN with an activity of 1 mC was used in the present synthesis. The high specific activity of the cyanide made it possible to dilute considerably the radioactive sugars with inactive material since only a specific activity of approx. 1 μC per milligram was desired.

Isbell *et al.* discovered, when synthesising labelled glucose and mannose from D-arabinose, that the ratio of the epimeric aldonic acids depended upon whether the cyanohydrin reaction was acid catalyzed (predominantly mannonic acid) or base catalyzed (predominantly gluconic acid). We attempted similar conditions in the cyanohydrin synthesis starting from D-erythrose, but in both

cases arabonic acid was formed in a high proportion. To obtain a mixture with a higher proportion of the ribonic acid we decided to induce epimerization of the arabonic acid by the method of Fischer [3,4] which involves heating of the latter with pyridine. Unfortunately the degree of epimerization was not as high as hoped for, only approx. 20 %.

EXPERIMENTAL

Preparation of D-*erythrose.* The D-erythrose employed was prepared from 4,6-diethylidene-D-glucose by the method of Rappaport and Hassid [5]. The product was a slightly yellow syrup and had $[\alpha]_D^{20} -19.8°$ (water, *c* 6.86).

Addition of $K^{14}CN$ *to* D-*erythrose.* The method used was a modification of that reported by Isbell *et al.*[1] Potassium hydroxide (11 mg) and $K^{14}CN$ (20.6 mg, 1 mC, containing 9 mg potassium hydroxide) were dissolved in water (3 ml) in a 50 ml flask fitted with a long neck and a quick fit stopper. The mixture was frozen in a mixture of acetone and solid carbon dioxide. D-Erythrose (80 mg) dissolved in water (1 ml) was added and subsequently solid carbon dioxide (160 mg). The stopper was kept in position and the mixture allowed to thaw. When melted the stopper was removed for a moment to release the pressure. The flask was kept at 0° C for 20 hours and then at room temperature for two days. The mixture was then heated to 50° C while bubbling air through the solution. To trap any radioactive HCN, the escaping air was passed through a solution of KOH. The reaction mixture was then concentrated to dryness *in vacuo* in the solid state. The residue was dissolved in water (1 ml) and ethanol (15 ml) added and the mixture put aside at 0° C for 4 days. Crystalline plates of the potassium salts of the aldonic acids together with a small amount of syrupy material had precipitated. The supernatant was easily decanted off and the crystals washed three times with alcohol (10 ml each time) and finally three times with ether (10 ml each time). The last traces of ether were removed in a desiccator.

Epimerization of arabonic acid. The crystalline mixture of the potassium salts of the aldonic acids was dissolved in water (30 ml) and passed slowly through a column of Amberlite IR-120(H) (7 ml wet resin, hydrogen form) to convert the salt to the free acids. The column was washed with water until negligible activity appeared in the eluate. The solution was concentrated to dryness in the solid state *in vacuo*. The residual syrup was dissolved in a small volume of pyridine (0.07 ml) and transferred to a pyrex tube (diameter 1.5 cm, length 10 cm). The flask was washed out four times with water (0.25 ml each time). The tube was sealed and heated at 130° C for two days. The dark content of the tube was diluted with water (10 ml), passed through a column of Amberlite IR-120(H) (4 ml wet resin, hydrogen form) and the column washed until negligible activity appeared in the eluate. The slight colour of the eluate was removed by treatment with charcoal. The filtrate was evaporated to dryness in the solid state *in vacuo* to yield a straw coloured syrup.

Lactonization of the aldonic acids. To induce lactonization the mixture of the aldonic acids was heated at 90° C for one hour *in vacuo* (0.01 – 0.001 mm Hg).

Reduction of the lactones with sodium amalgam. To ensure a suitable pH during the reduction we have used the mixture of oxalic acid and sodium oxalate introduced by Isbell and collaborators [1] for the reduction of the lactones of gluconic and mannonic acids. As suggested by these workers we also used a 5 % sodium amalgam. We encountered, however, difficulties in preparing a sodium amalgam which would give us a constant yield of the pentoses. Judging from trial experiments using inactive material the yield of the pentoses seemed to depend upon the quality of the amalgam. In one reduction we would obtain the usual yield of the sugars and with a new batch of sodium amalgam practically nothing. It seems to us that an amalgam with poor quality is obtained if the temperature during its preparation is too high. In our experience the temperature must not be so high as to give a dark surface on the amalgam. Thus when preparing 5 % sodium amalgam we proceeded as follows*: A 3 % amalgam was prepared by adding

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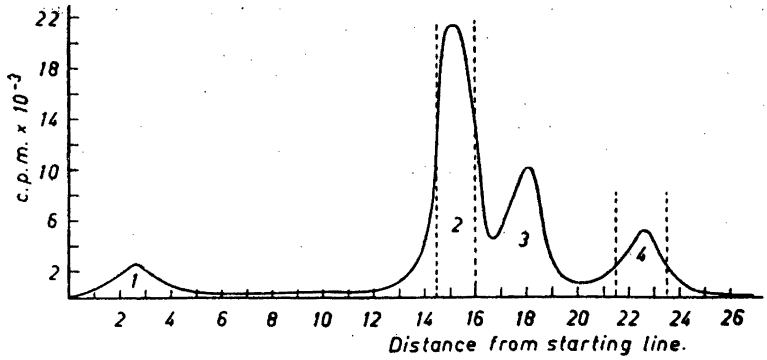

Fig. 1. The distribution of radioactivity along the paper chromatogram. Peak 2 and 4 are arabinose and ribose respectively.

sodium to the pure mercury, the amalgam was subsequently slightly heated and further sodium added. The mixture was then heated under stirring until just melted and poured into oil.

The reduction was carried out in a tube (diameter 2 cm, length 10 cm) fitted with a glass stopper which contained a small hole for the hydrogen to escape. The tube was immersed in a mixture of ice and water. The mixture of the lactones was dissolved in icecold water (3.5 ml) and transferred to the tube. Oxalic acid (117 mg) and sodium oxalate (133 mg) were added. Sodium amalgam (800 mg, 5 %) was then added and the mixture shaken for three hours in the cooling mixture, and left at room temperature over night. To remove all ions the reaction mixture was passed through a small coloumn of Amberlite IR-120(H) (2.5 ml wet resin) and subsequently through a column of Amberlite IR-4B (OH) (2.5 ml wet resin). The coloumns were washed with water until negligible activity was present in the eluate. The amounts of pentoses was determined in the eluate using the colourimetric method of Euler and Hahn [6]. The eluate contained 11.4 mg and 9.5 mg when calculated as arabinose and ribose respectively. This corresponded to a yield of approx. 25 % when calculated from the amount of K^{14}CN used.

Chromatographic isolation of D-*ribose-1-*^{14}*C and* D-*arabinose-1-*^{14}*C.* The eluate containing the two pentoses was concentrated to dryness *in vacuo* in the frozen state. The residue was dissolved in a small volume of water (0.4 ml). An aliquot (0.1 ml) was put on a sheet (25 cm × 55 cm) of Whatman paper No. 3 in a band of length 15 cm and width 2 mm. On both sides of the band was put a mixture of inactive ribose and arabinose as reference compounds. The paper chromatogram was run for three days in the top layer of a mixture of water-butanol-ethanol-ammonia (49:45:5:1, v/v/v/v). The reference runs were developed with aniline hydrogene phthalate and used as a guide when locating the positions of the radioactive sugars. A very narrow strip (0.5 cm) was cut out of that part of the paper which contained the radioactive run, cut transversely in small segments (0.5 cm width) and the activity of the segments determined using a thin window Geiger Müller tube. The distribution of the activity along the chromatogram is seen on Fig. 1. Peak 2 is arabinose and peak 4 is ribose. The other two peaks are unidentified. The areas on the chromatograms containing the radioactive sugars were cut out, these are indicated between the dotted lines in Fig. 1. It was necessary to run three more chromatograms to separate the remaining of the mixtures of the pentoses. The radioactive sugar strips were eluted with water and the solutions concentrated to dryness in vacuum in the frozen state, yielding small amounts of white material. The amounts of the pentoses were determined according to the method of Euler and Hahn [6]. The yields were:

D-arabinose-1-^{14}C 6 mg and D-ribose-1-^{14}C 1.8 mg.

The purity of the sugars was tested by running paper chromatograms in different solvent mixtures and examining the radioactivity along the chromatograms as described

above. The arabinose was homogeneous in the following solvent systems: butanol-acetic acid-water (4:1:5, v/v/v) and phenol saturated with water. It had an activity of 3.24×10^6 c.p.m. per milligram. Ribose when examined in the butanol acetic acid solvent contained 5—6 % of impurities. The radioactive ribose was therefore further purified by paper chromatography in this solvent system and the ribose eluted again from the chromatogram. It was only necessary to run one chromatogram to achieve this. The purified ribose-1-^{14}C was homogeneous in the phenol water solvent system. It had an activity of 3.07×10^6 c. p. m. per milligram. The sugars were plated directly and counted using the thin window Geiger Müller tube.

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