Preparation of 2-(Acetamido-1-^{14}C)-2-Deoxy-D-Glucose, 2-(Acetamido-1-^{14}C)-2-Deoxy-D-Galactose and L-Iduronic Acid-6-^{14}C

Received January 31, 1970.

With the ever increasing interest being taken in the biosynthetic pathway to the acidic mucopolysaccharides and other uronic acid- and 2-acetamido-2-deoxy-D-hexose-containing naturally occurring macromolecules, there is a need for readily available labelled precursors. Some of these are commercially available. Simple methods of preparation of three sugars in forms suitable for biosynthetic investigations are now reported.

2-(Acetamido-1-^{14}C)-2-deoxy-D-glucose and 2-(acetamido-1-^{14}C)-2-deoxy-D-galactose.

The original method of N-acetylating the parent 2-amino-2-deoxysugar [1] has been scaled down and adapted. (Acetic-1-^{14}C)-anhydride (15 mCi/mmole, 50 μCi), obtained from The Radiochemical Centre, Amersham, U.K., was diluted on a vacuum apparatus with non-labelled acetic anhydride to 180 μl, and a solution of the 2-amino-2-deoxy-D-hexose hydrochloride (300 mg) in water (6.9 ml), and methanol (0.7 ml) were added. The mixture was stirred for 1.5 hours at 0-5 °C with Dowex 1X8 ion exchange resin, 20-50 mesh, carbonate form, and the solution filtered. The combined filtrate and washings were passed through a column of AG 1X8 ion exchange resin, 100-200 mesh, hydrogen form (5 ml) to absorb any unconverted 2-amino-2-deoxy-D-hexose. The combined eluate and washings were concentrated by rotary evaporation *in vacuo* (bath temperature 40 °C), and the syrupy residues crystallised on standing. The chemical and radiochemical purity of the prepared labelled sugars were established by paper chromatography using a solvent system [2] of *n*-butanol/pyridine/water, 6 : 4 : 3 by volume and Whatman No. 1 paper. After development and drying, autoradiography was carried out using Kodirex X-ray film, followed by spraying with silver nitrate. Both the silver and autoradiograph spots derived from the prepared labelled sugars coincided with the silver spots from appropriate unlabelled standards. None of the starting materials were identified in the sugar preparations. The specific activities were determined using the scintillation medium described elsewhere [3] (2-(acetamido-1-^{14}C)-2-deoxy-D-glucose, 8.0 μCi/mmole; 2-(acetamido-1-^{14}C)-2-

deoxy-D-galactose, 10.6 μCi/mmole). Clearly this method can be applied without modification to the labelling of the acetyl group, not only of any N-acetylated sugar, but also to other N-acetylated compounds.

L-Iduronic acid-6-^{14}C.

Barium 1,2-*O*-isopropylidene-α-L-iduronate-6-^{14}C (5 mg) obtained from The Radiochemical Centre was dissolved in water (250 μl) and stirred with a solution of oxalic acid (100 mg) in water (250 μl) for 1.5 hours at 100 °C, to remove the barium ion and isopropylidene group [4]. Decolourising charcoal was added for the last 0.5 hour. The resultant mixture was filtered from barium oxalate and charcoal, and rotary evaporated *in vacuo* (bath temperature 40 °C) to a syrup which subsequently crystallised. This L-idurono-6,3-lactone may be used in solution as the lactone or as the corresponding salt according to the pH of the solution, but it usually contains up to 2 % of the D-*gluco*-isomer as impurity. The product may be purified by loading it as a solution in 0.2 M sodium acetate (2 ml) on to a column of AG 1X2 ion exchange resin, 200-400 mesh, acetate form (28 ml) and fractionating [4] using 0.1 M sodium acetate as eluent. Fractions (5 ml) were collected and assayed [3] for radioactivity; the scan showed a major peak which contained the L-*ido*-isomer and an immediately preceding small peak which contained the D-*gluco*-isomer.

Acknowledgements.

The author gratefully acknowledges the advice of Dr S. A. Barker D. Sc. and the financial support of the Medical Research Council.

J. F. KENNEDY,

Department of Chemistry, University of Birmingham,
Birmingham 15, England.

REFERENCES

1. ROSEMAN, S. and LUDOWIEG, J. — *J. Am. Chem. Soc.*, **76** : 301 (1954).
2. CRUMPTON, M. J. — *Biochem. J.*, **72** : 479 (1959).
3. KENNEDY, J. F. — *Experientia*, **25** : 1120 (1969).
4. DZIEWIATKOWSKI, D. — *Biochim. Biophys. Acta*, **56** : 167 (1962).