AAEC/E440

AAEC/E440

AUSTRALIAN ATOMIC ENERGY COMMISSION

RESEARCH ESTABLISHMENT

LUCAS HEIGHTS

THE SYNTHESIS OF 4-^{14}C-PYRIDINE-2,6-DICARBOXYLIC ACID

by

J. ROBSON
P.J. SORBY

February 1978

ISBN 0 642 59641 7

AUSTRALIAN ATOMIC ENERGY COMMISSION
RESEARCH ESTABLISHMENT
LUCAS HEIGHTS

THE SYNTHESIS OF 4-^{14}C-PYRIDINE-2,6-DICARBOXYLIC ACID

by

J. ROBSON
P.J. SORBY

ABSTRACT

Dipicolinic acid (pyridine-2,6-dicarboxylic acid), labelled with ^{14}C, has been synthesised, on a miniature scale, starting from formaldehyde-[^{14}C]. The radioactive label was incorporated into the '4' position of the pyridine ring to prevent possible loss of label owing to decarboxylation during experiments. The compound was intended for use in the localisation of dipicolinic acid in bacterial spores.

National Library of Australia card number and ISBN 0 642 59641 7

The following descriptors have been selected from the INIS Thesaurus to describe the subject content of this report for information retrieval purposes. For further details please refer to IAEA-INIS-12 (INIS: Manual for Indexing) and IAEA-INIS-13 (INIS: Thesaurus) published in Vienna by the International Atomic Energy Agency.

PICOLINIC ACID; CARBON 14 COMPOUNDS; LABELLING; BACTERIAL SPORES; FORMALDEHYDE; CHEMICAL PREPARATION

CONTENTS

1. INTRODUCTION

Dipicolinic acid (pyridine-2,6-dicarboxylic acid) is known to occur in bacterial spores [Powell 1953]; to pursue certain bacteriological investigations, this compound was required in a radioactively labelled form [Germaine & Murrell 1974]. Although dipicolinic acid labelled with tritium was commercially available this was not the case for the carbon-14 labelled compound. The established methods of synthesis leading to dipicolinic acid were therefore examined to assess whether they could be applied to the preparation of the carbon-14 labelled compound.

The method frequently employed for the preparation of pyridine carboxylic acids has been the oxidation of a methyl pyridine [Black *et al.* 1949; Soine & Buchdahl 1950; Vasa *et al.* 1968], although oxidation of a pyridine aldehyde [Klosa 1955] has also been used. Hence, to obtain dipicolinic acid the obvious route was *via* the oxidation of 2,6-dimethyl-pyridine (2,6-lutidine) for which there was an established synthetic method [Singer & McElvain 1955]. The sequence of reactions shown in Figure 1 appeared to be most suitable for the preparation of the carbon-14 labelled compound because it allowed the label to be introduced into a ring position from the relatively low cost intermediate, formaldehyde-[^{14}C]. It was also considered that the label in the ring position would be less likely to be lost if the product was used in experimental systems where decarboxylation reactions could occur.

2. EXPERIMENTAL

The following sequence of reactions leading to dipicolinic acid is shown in Figure 1.

2.1 Synthesis of 4-^{14}C-2,6-Dimethyl-Pyridine

Two millicuries (74 MBq) of formaldehyde-[^{14}C] at 17 mCi (629 MBq) mmol^{-1} (the Radiochemical Centre, Amersham, UK) was washed into a 50 mℓ centrifuge tube from its glass ampoule pack with 4.5 mℓ of a 2 per cent wt/vol. aqueous solution of formaldehyde. Six hundred and fifty milligrams of freshly distilled ethyl acetoacetate was added, the mixture was cooled to 0°C in an ice bath and then 0.1 mℓ of diethylamine was added. The mixture was maintained at 0°C for 24 hours then allowed to stand at room temperature with occasional manual stirring for 48 hours. The upper aqueous phase was removed and extracted twice with 3 mℓ aliquots of diethyl ether. The ether extracts were combined with the lower organic phase and the mixture was dried over anhydrous sodium sulphate.

The dry solution was transferred to a clean centrifuge tube and the ether distilled off under a stream of nitrogen. The residue was dissolved in 3 mℓ of absolute ethanol and ammonia gas passed through the solution for four hours. Finally it was allowed to stand at room temperature for 48 hours, by which time the solvent had evaporated leaving slightly oily yellow crystals of 4-^{14}C-1,4-dihydro-3,5-dicarbethoxy-2,6-dimethyl-pyridine(I). These crystals were not purified but used directly in the next step in which 3.5 mℓ of a mixture of nitric acid:sulphuric acid:water (12:13:45 by weight) was added; the mixture was warmed cautiously over a water bath until the initial vigorous reaction subsided. Then it was maintained at 100°C for one hour and cooled to ice bath temperature.

Four grams of finely crushed ice were added and the solution was made alkaline with ammonia to precipitate 4-^{14}C-3,5-dicarbethoxy-2,6-dimethyl-pyridine(II) which was separated by centrifuge. The supernatant solution was discarded and the precipitate dried in a vacuum desiccator. The dry residue, remaining in the centrifuge tube was dissolved in 1 mℓ of ethanol and 1 mℓ of ethanolic potassium hydroxide (20 per cent wt/vol.) added. After refluxing for one hour, the solvent was allowed to evaporate and the residue containing the potassium salt of 4-^{14}C-3,5-dicarboxy-2,6-dimethyl-pyridine(III) was dried in a vacuum desiccator. The dry material was ground with 2 g of dry calcium oxide and the mixture transferred to a retort of fused silica. An additional 0.5 g of calcium oxide was used to scrub the remaining traces of dry residue from the centrifuge tube and added to the contents of the retort. On heating to bright redness, 4-^{14}C-2,6-dimethyl-pyridine(IV) was distilled and collected in a 20 mℓ flask. When distillation was complete, the retort was allowed to cool and 5 mℓ of water added. It was reheated to distil over the residual traces of product into the receiver.

2.2 Preparation of 4-^{14}C-Pyridine-2,6-Dicarboxylic Acid

The aqueous solution of 4-^{14}C-2,6-dimethyl-pyridine was diluted to approximately 12 mℓ with distilled water, 500 mg of finely ground potassium permanganate was added and the solution was refluxed for 48 hours. Constant mechanical stirring was essential throughout the period to avoid the bumping caused by the formation of manganese dioxide during the course of the reaction. After cooling, drops of hydrogen peroxide solution (30 per cent) were added to the solution to decompose the residual permanganate, then it was boiled to destroy the excess of peroxide. The manganese dioxide precipitate was filtered off and the

filter cake washed with boiling water (approximately 250 mℓ was required) until the filtrate was neutral to pH indicator paper. The filtrate and washings were combined and evaporated to 1 mℓ containing the product as 4-^{14}C-2,6-pyridine dicarboxylic acid dipotassium salt.

2.3 Analysis of Product

2.3.1 Identification

The melting point of a non-radioactive preparation of the free acid synthesised by this method was 249-251°C (decomposed) which corresponded favourably with the reported value of 252°C [Heilbron & Bunbury 1953]; no depression in the melting point was observed for an equal parts mixture with an authentic sample of dipicolinic acid.

The ultraviolet absorption spectrum of a 1:100 solution of the radioactive preparation corresponded with one obtained from an authentic sample, and the R_f value on a thin layer chromatogram also corresponded to the value for the authentic material.

2.3.2 Quantitative estimation

A small aliquot of the solution of dipotassium dipicolinic acid-[4-^{14}C] was diluted accurately to 100 times the volume with water and the absorbance at 265 nm measured against an air blank. The concentration, calculated from a calibration graph obtained with the non-radioactive material, corresponded to a yield of 50 mg of dipicolinic acid. This represents an overall 12 per cent yield (based on formaldehyde) from the synthesis.

2.3.3 Radioactive concentration

The radioactive concentration of the dipotassium dipicolinic acid-[4-^{14}C] solution was determined by counting a small aliquot of the 100 times dilution (see Section 2.3.2) in a liquid scintillator both alone and with the addition of an aliquot of formaldehyde-[^{14}C] solution which had been calibrated by the AAEC Radioisotope Standards Group. The total activity of the preparation was then calculated to be 290 μCi (10.7 MBq).

2.3.4 Radiochemical purity

Thin layer chromatography of the radioactive preparation on silica gel plates (Eastman No.6061), with acetic acid:methanol (3:1) as the mobile phase, followed by the liquid scintillation counting of small sections of the chromatogram revealed the presence of two radioactive areas. By comparison with the non-radioactive compounds the major one, at R_f 0.47, was found to correspond to 2,6-pyridine dicarboxylic acid

and the minor one, at R_f 0.63, to 2-pyridine carboxylic acid. With diethylamine:ethylacetate:water (2:2:1) as the mobile phase, two radioactive species were again found, the major one at R_f 0.12 and the minor at R_f 0.55 corresponding to the di and monocarboxylic acids respectively.

Quantitative results obtained by counting the chromatograms with a liquid scintillator showed 95 per cent of the radioactivity to be associated with the 2,6-pyridine dicarboxylic acid and 5 per cent with the monocarboxylic acid. In all cases, a small radioactive peak was found at the origin; this was also the case with an authentic, non-radioactive sample of dipicolinic acid.

3. DISCUSSION

The problems to be overcome in this preparation arise because of the overriding requirements of radioactive labelling in a normally straightforward multistep synthesis. As the ^{14}C label was to occupy a ring position, and its introduction was the first step in the preparation, a sufficient quantity of the radioactive starting material had to be provided to compensate for the progressively reduced yield at each subsequent step. The overall yield for this synthesis, calculated from the yields for each step given in the references was 17 per cent at best.

It was important in this particular application [Germaine & Murrell 1974] for the specific activity of the labelled dipicolinic acid to be sufficiently high to determine minute traces of the labelled compound in experimental systems by measurement of the associated radioactivity. The relatively simple modifications to the standard experimental technique have enabled the reactions to be carried out on a miniature scale without significantly lowering the yield. In this way, a relatively small quantity of the labelled dipicolinic acid was prepared at a specific activity of 1 mCi (37 MBq) $mmol^{-1}$ which was adequate for the application and, thereby, the optimum use was made of the formaldehyde-[^{14}C], the most costly of the raw materials.

4. REFERENCES

Black, G., Depp, E. & Corson, B.B. [1949] - Oxidation of Certain Methyl Pyridines to Pyridine Carboxylic Acids. *J. Org. Chem.*, 14 : 1421.

Germaine, G.R. & Murrell, W.G. [1974] - Use of Ultraviolet Radiation to Locate Dipicolinic Acid in *Bacillus cereus* Spores. *J. Bacteriol.*, 118 : 202.

Heilbron, I. & Bunbury, H.M. [1953] - Dictionary of Organic Compounds, Eyre & Spottiswoode, London.

Klosa, J. [1955] - The Oxidation of Pyridine Aldehydes. *Arch. Pharm.*, 288 : 426.

Powell, J.F. [1953] - Isolation of Dipicolinic Acid (Pyridine-2,6-Dicarboxylic Acid) from the Spores of *B. megaterium*. *Biochem. J.*, 54 : 210.

Singer, A. & McElvain, S.M. [1955] - 2,6-Dimethyl-Pyridine. *Organic Syntheses Coll.* Vol.3, p.740. John Wiley & Sons Inc., New York.

Soine, T.O. & Buchdahl, M.R. [1950] - A Note on the Preparation of Dipicolinic Acid. *J. Am. Pharm. Assoc.*, 39 : 421.

Vasa, V.B., Parmaeswaran, V.K., Khoranc, M.L. & Tipnis, H.P. [1968] - Synthesis of 6-[3-(Acetoxymercuri)-2-Hydroxypropyl-Carbarmyl]-Picolinic Acid. *Indian J. Pharm.*, 30 : 252.

NOTES

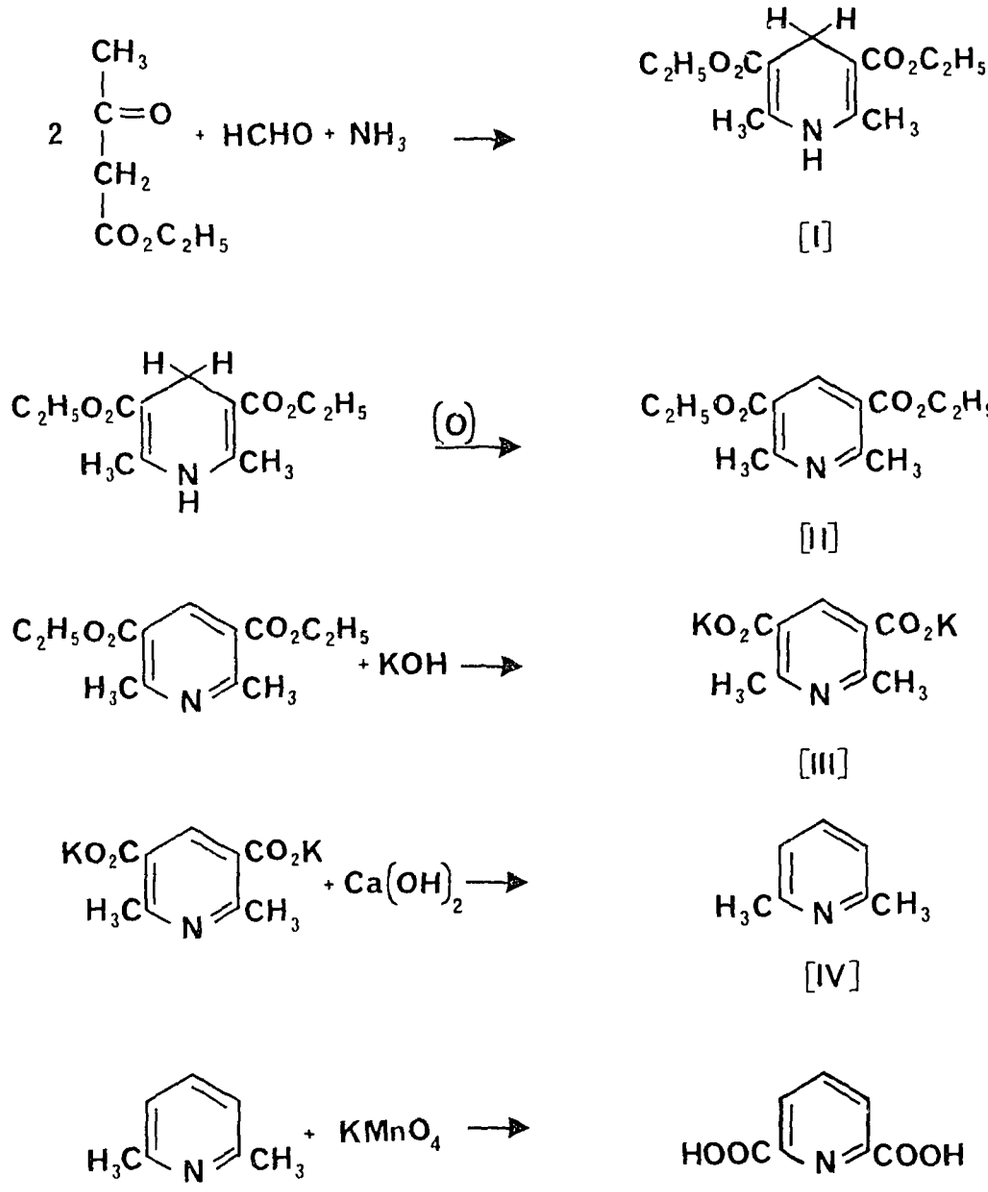

FIGURE 1. THE SYNTHESIS OF PYRIDINE-2,6-DICARBOXYLIC ACID

Printed by the AAEC Research Establishment