# A SIMPLE SYNTHESIS OF 2,6-DIAMINOPIMELIC-/ 1,7-$^{14}$C/ ACID

A.Arendt, A.Kolodziejczyk
Department of Organic Chemistry
Institute of Organic and Food Chemistry
and Technology
Technical University
Gdańsk - Poland
Received on January 30, 1973

## SUMMARY

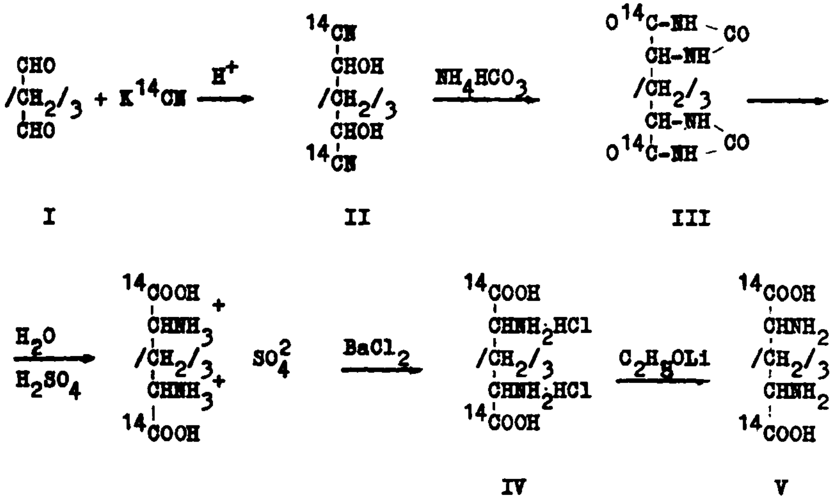
*A convenient method of synthesis of 2,6-diaminopimelic / 1,7-$^{14}$C / acid / DAP / has been elaborated. Glutaric aldehyde and $K^{14}CN$ produced the dicyanohydrine which was converted without izolation into the dihydantoine derivative of DAP by treatment with $NH_4HCO_3$. Hydrolisis / $H_2SO_4$ / and removal of sulphate ions gave the crystalline, chromatographically pure DAP / 1,7-$^{14}$C / of spec. activity 4,42 mCi / mmole. The radiochemical yield of the synthesis was 66%.*

## INTRODUCTION

2,6-Diaminopimelic acid /DAP/ is the component of the muropeptides, the essential constituents of the cell walls of several microorganisms. Since the biosynthesis of cell wall and the mechanisms of the action of the antibiotics inhibiting this biosynthesis are the subject of intensive studies, the demand for a radioactive DAP, as a precursor of muropeptides, is still increasing.

The hitherto reported methods of preparation of $^{14}$C labelled DAP involve multi-steps procedures which use expensive, radioactive substrates and result in low yields of the product. /2-$^{14}$C/ DAP was prepared from /2-$^{14}$C/ acetamidomalonate [1,2,3]; whereas the classical bromination and amination with succin - imide of the pimelic /1,7-$^{14}$C/ acid gave the /1,7-$^{14}$C/ DAP [5,6].

The method reported here, based on the patent [7,8,9] procedures of preparing the non-radioactive DAP from glutaric aldehyde and hydrogen cyanide, is simple, effective and involves the use of the readily available K$^{14}$CN. The mmole scale of the synthesis made necessary to introduce some modifications to the procedure. Potassium cyanide was used instead of HCN and the cyanohydrine formed was converted in aq. ammonium carbonated into the dihydantoine without any isolation.



After hydrolysis of the hydantoine in sulphuric acid and removal of the sulphate ions by treatment with BaCl$_2$, the /1,7-$^{14}$C/ DAP hydrochloride was obtained. After neutralization with lithium ethanolate it produced free, crystalline and chromatographically pure /1,7-$^{14}$C/ DAP, of spec. activity 4.42 mCi/

**mmole. The yield of the synthesis /based on glutaric aldehyde/ was 74%; the radiochemical yield /based on K$^{14}$CN/ was 66%.**

## Experimental

**Chromatography, activity measurement.**
**Chromatographic control was carried out by means of the TLC method, using Kieselgel G /E. Merck/; ethyl acetate was employed as a solvent and iodine vapours as a developing agent. Radioactivity was measured with a liquid scintillation scanner Isocap/300 /Nuclear-Chicago/.**

**2,6-Diaminopimelic acid /1,7-$^{14}$C/.**

**The commercial /Fluka AG/ glutaric aldehyde /150 mg; 1,5 mmole, as a 25% aq. solution/ and 2 ml of 2 N Hydrochloric acid were placed in the thick-glass, round-bottom 25 ml flask, equipped with a magnetic stirrer. To this solution K$^{14}$CH /108 mg; 1.66 mmole; spec. acivity 4.5 mCi/mmole; total activity according to the certificate was 7.5 mCi/ was added slowly with stirring, and the potassium cyanide ampoule was washed with water /2 x 0.5 ml/ and the washings were added to the reacion mixture. After further 5 min. non-radioactive KCN /87 mg; 1.34 mmole/ was added, the solution was then neutralized with pyridine up to pH = 6 and stirred for 45 min.**

**The TLC control showed that only single product of $R_f$ = 0.9 was present, containing the total readioactivity.**

**$NH_4HCO_3$ /1.6 g; 20 mmole/ and 25% aq. $NH_3$ /3 ml/ were added, the flask was stoppered firmly with the polyethylene stopper and heated at 95° for three hr. TLC showed that the radioactivi-**

ty-containing product remained exclusively at a starting point of the chromatogram. The volatile components were evaporated under reduced pressure, 15 ml of benzene was added and evaporated again. The hydantoine was extracted from the residue with three portions of methanol /10, 5,5 ml/ and the combined extracts were evaporated under reduced pressure. 65% sulphuric acid /0.87 ml/ was then added and the mixture was heated at 150° for 4 hr. After cooling, 5 ml of water and 10 ml of 1.8 N /9 mmole/ $BaCl_2$ solution was added, boiled with a small amount of the charcoal, filtered, washed with 5 ml of water and the combined filtrates were evaporated in vacuo. 5 ml of water was added to the residue, evaporated, then added 15 ml of benzene and evaporated again. The obtained hydrochloride of /1,7-$^{14}C$/ DAP, contaminated with some ammonium chloride, was extracted with hot, absolute ethanol /3 x 5 ml/; 2 ml of water was added to the combined extracts and the solution was neutralized with lithium ethanolate up to pH = 6.4. DAP separated as an oil, which solidified after cooling. The solution was decanted, the crude product dissolved in 1 ml of water and precipitated by addition of 5 ml of ethanol. The chromatogaphically pure /1,7-$^{14}C$/ DAP was filtered off; yield 213 mg /74.8% based on glutaric aldehyde/; total activity was 4.95 mCi which corresponded to 66% of the radiochemical yield.

## REFERENCES

1. Veres K., Chaloupka J. Use Radioisotopes Animal Biol. Med. Sci. Proc. Conf.Mexico City 211 /1961/.

2. Veres K., Benes J., Hanus J.
Prochazkova V. Prepn. Bio-Med.Appl.Labelled.Mol. Proc. Symp. Venice 67 /1964/
3. Hanus J., Veres K. J. Label Compounds 6 153 /1970/
4. Finlayson A.J.,
Simpson F.J. Can.J.Biochem. and Physiol. 39 1551 /1961/
5. Akabori S., Izumi Y.
Fujiwara F. J. Chem.Soc. Japan 75 993 /1954/
6. Tannenbaum S.W., Kanako K. Biochemistry, 3 1314 /1964/
7. Emmick R.D., Londergan T.E. Brit. 875, 353; Chem. Abs. 57 5805d /1972/
8. Galloway E.C. US 3, 122, 583; Chem. Abs. 60 13145c /1964/
9. Hause N.L. US 3, 136, 775; Chem. Abs. 61 436h /1964/