AN IMPROVED SYNTHESIS OF CARBON-14 LABELLED CARBOXYLIC ACIDS FROM CARBON-14 LABELLED AMINO ACIDS

T.V. Ramamurthy, S. Ravi and K.V. Viswanathan
Labelled Compounds Section, Isotope Group
Bhabha Atomic Research Centre, Trombay, Bombay 400085

SUMMARY

Various Carbon-14 labelled amino acids including the aromatic ones viz., tyrosine, phenylalanine and tryptophan are converted to the corresponding carboxylic acids in high yield (70 -90%) on a micromolar scale synthesis by reaction with hydroxylamine-O-sulphonic acid and in a short reaction time. The improvement in yield has been achieved by using aqueous alcohol as solvent in lieu of water alone as the medium of reaction.

Key words : Hydroxylamine-O-sulphonic acid, amino acids-^{14}C(U), tryptophan-carboxyl-^{14}C, 3-indolylpropionic acid-carboxyl-^{14}C, 3-phenylpropionic acid-^{14}C(U), 3-(4-hydroxyphenyl) propionic acid-carboxyl-^{14}C.

Carbon-14 labelled amino acids are convenient starting materials for the preparation of useful labelled compounds. We have shown the utility of hydroxylamine-O-sulphonic acid (HAOS) as an efficient reagent for the small scale synthesis of a few carboxylic acids-^{14}C(U) from α-amino acids-^{14}C(U)[1]. Uniformly ^{14}C labelled compounds retaining the same carbon skeleton of the original amino acids can thus be synthesised with high specific activity. The reaction conditions reported for non-radioactive synthesis were slightly changed[2], in that a large excess of HAOS (20 fold) was employed. When we attempted to extend the method to aromatic amino acids, we noticed that the yields of the products reached a maximum of around 50% even though excess reagent, higher temperature (100°C)

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and longer reaction time were used. The preparation of carboxylic acids from ^{14}C labelled aromatic amino acids by HAOS aroused our interest as the acids like 3-indolylpropionic acid (using tryptophan as substrate) and 3-(hydroxyphenyl) propionic acid were difficult to synthesize by other methods[4]. Further, the literature yields of carboxylic acids from various aromatic amino acids with diverse groups on the benzene ring were low (21-45%), thus limiting the usefulness of the method[2]. Literature references on the use of HAOS for several other reactions had employed methanol as a solvent. We, therefore, decided to study the efficacy of using various aqueous solvent combinations from the point of maximizing the yield of microscale synthesis of carboxylic acids from amino acids. We settled on this combination because the reagents, namely amino acids, potassium hydroxide and to a lesser extent HAOS have low or poor solubility in organic solvents alone. Besides methanol, other solvents tried were ethanol, dimethylsulphoxide, dimethylformamide, methylcellosolve, dioxane, etc.

The reaction consisted of simply heating a mixture of the amino acid, HAOS and potassium hydroxide in the appropriate water - organic solvent mixture under reflux for 30 minutes. Initially, the reaction was studied using tracer amounts of tryptophan-carboxyl-^{14}C or phenyl alanine-^{14}C(U). The reaction mixture was analysed for the formation of carboxylic acids by TLC. TLC or paper chromatography with radioactivity scanning was fast enough to reveal the extent of the progress of the reaction. The counting of ether extract after acidification of reaction mixture gave a quantitative picture of the reaction. On the basis of the results (Table 1) obtained with tryptophan-carboxyl-^{14}C and phenylalanine-^{14}C(U), the solvent methanol : water (40 : 60) v/v was found to be an efficient medium for the reaction. The same conditions were found to be ideal for the other aromatic amino acid, tyrosine as well.

Table 1 : Solvent effects on HAOS reaction with tryptophan and phenylalanine.

No.	Solvent medium employed	Radiochemical yield (%) *	
		3-Indolylpropionic acid-carboxyl-^{14}C	3-Phenylpropionic acid-^{14}C(U)
1.	Water	40	25
2.	Water : monoglyme (50:50, v/v)	56	60
3.	Water : dioxane (50:50, v/v)	24	63
4.	Water : methyl cellosolve (50:50, v/v)	57	60
5.	Water : dimethyl sulphoxide (50:50, v/v)	5	45
6.	Water : dimethylformamide (50:50, v/v)	7	5
7.	Water : methanol (60:40, v/v)	90	95
8.	Water : ethanol (60:40, v/v)	90	95

*In all the above experiments the labelled amino acid (50 μmoles, 10 μCi) in excess alkali (2 mmoles) was treated with HAOS (1 mmole) under reflux for 30 minutes. The radiochemical yields were measured after isolation.

It was thus seen that high yields could be obtained when aqueous methanol or ethanol was used as a solvent. Hence the reaction was repeated with aliphatic amino acids and in every case the carboxylic acid could be isolated in high yield. The procedures reported earlier were adopted for the purification and isolation of the acids[1]. The results obtained in this manner along with results of the reaction conducted in aqueous medium are presented in Table 2.

Table 2 : Synthesis of ^{14}C labelled carboxylic acids from ^{14}C labelled amino acids in water and aqueous alcohol.

No.	Amino acid used	Product	Radiochemical yield (%) * Water	Water:alcohol
1.	Aspartic acid-^{14}C(U)	Succinic acid-^{14}C(U)	90	90
2.	Glutamic acid-^{14}C(U)	Glutaric acid-^{14}C(U)	80	80
3.	Threonine-^{14}C(U)	β -Hydroxybutyric acid-^{14}C(U)	55	90
4.	Valine-^{14}C(U)	Isovaleric acid-^{14}C(U)	65	70
5.	Phenylalanine-^{14}C(U)	3-Phenylpropionic acid-^{14}C(U)	25	94
6.	Tyrosine-^{14}C(U)	3(4-hydroxyphenyl) propionic acid-^{14}C(U)	50	73
7.	Tryptophan-carboxyl-^{14}C	3-Indolylpropionic acid-carboxyl-^{14}C	40	90
8.	Tyrosine carboxyl-^{14}C	3-(4-hydroxyphenyl) propionic acid-carboxyl-^{14}C	50	73
9.	Phenylalanine-carboxyl-^{14}C	3-phenylpropionic acid-carboxyl-^{14}C	25	95

* In the above experiments the quantity of amino acids employed was maintained at 50 μmoles whereas the radioactivity varied from 20 to 100 μCi. The yields were the average values of three experiments.

EXPERIMENTAL

Amino acids-^{14}C(U) isolated from algae grown by photosynthesis in $^{14}CO_2$ atmosphere were exhaustively purified and used[7]. Tyrosine-carboxyl-^{14}C, phenylalanine-carboxyl-^{14}C and tryptophan-carboxyl-^{14}C were prepared in our laboratory by Strecker method using alkali cyanide-^{14}C as the starting material[8,9].

Synthesis of 3-indolylpropionic acid carboxyl-^{14}C (IPA) :

To a round bottom flask (50 ml) containing tryptophan (10 mg, 50 μmmole), the labelled tryptophan (10 μCi) was added as an aqueous solution (1 ml). Potassium hydroxide (2 ml, 1 N) was added to the flask which was cooled in an ice bath. Hydroxylamine-O-sulphonic acid (110 mg, 1 mmole) was weighed in a dry box and added. The solvent (water : methanol, 2 : 3 v/v 12 ml) was added to the reaction mixture and the solution was heated under reflux for 30 minutes. Analysis of the reaction mixture was carried out on an aliquot by TLC in the solvent system chloroform : acetic acid (95 : 5 v/v). This solvent system separated the amino acid and carboxylic acid which have a widely differing Rf. The product was revealed by its reaction with iodine vapour. The radioactivity scanning of TLC indicated the formation of IPA (Rf 0.9) with very little tryptophan (Rf 0.0) remaining unreacted. The reaction mixture was then acidified with dilute sulphuric acid and extracted with 3 x 10 ml portions of ether. The ether extracts were combined and counted; 9 μCi, radiochemical yield, 90 %. Analysis of the ether extract by TLC in the following solvents followed by radioactivity scanning showed IPA to be radiochemically pure and coincided with authentic IPA. They are visualised by both iodine and bromocresol green.

(1) Isopropanol : ammonia : water (8 : 1 : 1).

(2) n-Butanol : acetic acid : water (4 : 1 : 5).

Following the same procedure the other acids namely, 3-phenylpropionic acid-^{14}C(U), 3-phenylpropionic acid-carboxyl-^{14}C, 3-(4-hydroxyphenyl) propionic acid-^{14}C(U) and 3-(4-hydroxyphenyl) propionic acid carboxyl-^{14}C were synthesised. The compounds had UV spectra matching with authentic compounds.

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