^{11}C-LABELLED RADIOPHARMACEUTICALS: SYNTHESIS AND HIGH PRESSURE LIQUID CHROMATOGRAPHY OF NICOTINIC-^{11}C ACID AMIDE

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SUMMARY

Starting with $^{11}CO_2$, [carboxyl-^{11}C]-nicotinic acid amide was synthesized via 3-pyridyl-lithium in practically carrier-free amounts within 60 minutes. Overall radiochemical yields between 20% and 30% were obtained. Identification and purification of the labelled product was carried out by high pressure liquid chromatography using water as eluent.

Key words: Radiopharmaceuticals, [Carboxyl-^{11}C]-Nicotinic Acid Amide, High Pressure Liquid Chromatography

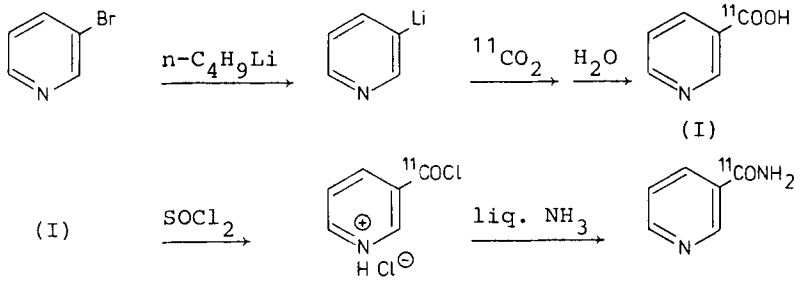
INTRODUCTION

Short-lived organic radionuclides such as nitrogen-13 (T = 10 min), carbon-11 (T = 20.4 min) and fluorine-18 (T = 110 min) are extremely well suited for in vivo studies of metabolic functions and for the determination of fast pharmacokinetics. Various ^{11}C-labelled compounds have been synthesized for nuclear medical applications (for reviews ref. cit. [1,2]). ^{11}C-labelled nicotinic acid has recently been prepared [3] to study its metabolism in vivo. Nicotinic acid and its amide are well known for their effect of drastically increasing capillary blood flow. Both acid and amide are thought to have similar vitamine activity and same physiological influence. For comparison

0362-4803/79/0216-0287$01.00

Received January 9, 1978

nicotinic acid amide was now labelled with ^{11}C in carrier-free amounts. Starting with $^{11}CO_2$ the following synthesis was worked out on the basis of classical synthetic steps:



The final radioanalytical quality control is carried out by means of radio high pressure liquid chromatography whose advantages have recently been reviewed [4].

EXPERIMENTAL

Production of carbon-11

Carbon-11 was produced at the Compact Cyclotron CV 28 via the $^{14}N(p,\alpha)^{11}C$ reaction, using a nitrogen gas target [5]. The experimental thick target yield was about 1.2 mCi $^{11}CO_2$/μA min and the radiochemical purity of the $^{11}CO_2$, checked by radio gas chromatography, was > 96%. For further synthesis $^{11}CO_2$ was used without any additional purification.

The $N_2(^{11}CO_2)$-stream coming from the target was carried into the adjoining laboratory via a gas loop and then trapped with liquid nitrogen. After a collection period of about 10 min

the trap was evacuated and the activity distilled into the reaction vessel at the same vacuum line. The reaction vessel was a small tube of 9 cm length and 0.8 cm i.d. containing a silicon rubber septum (thus allowing injection of the reactant solution with a hypodermic needle) and a small side arm with a teflon stop cock and a glass joint.

Synthesis

The synthesis of nicotinic acid amide was principally based on the method reported for carbon-14 labelling by Murray, Foreman and Langham [6]. Since ^{11}C-labelled nicotinic acid amide was to be prepared in carrier-free amounts the classical technique of reaction between stoichiometric amounts of the starting material could not be applied for this purpose, in addition fast purification methods had to be developed.

[Carboxyl-^{11}C]-nicotinic acid

[Carboxyl-^{11}C]-nicotinic acid was prepared by the reaction of 0.5 ml of 0.3 M 3-pyridyl-lithium in ether with $^{11}CO_2$. After hydrolysis with 1 ml H_2O the solution was extracted twice with 1 ml ether thus removing the excess of pyridine (for details see [3]), and then acidified with 0.1 ml 2 N HCl.

[Carboxyl-^{11}C]-nicotinoyl chloride hydrochloride

The solution containing the [carboxyl-^{11}C]-nicotinic acid was evaporated under vacuum to dryness in a 10 ml bulb at a rotation evaporator. 1 ml $SOCl_2$, freshly distilled, and 2 drops

of dimethylformamide were added. The mixture was refluxed for 10 min under vigorous stirring. The excess of $SOCl_2$ was removed at a second evaporator in a vacuum not below 20 torr to avoid sublimation of the product itself.

[Carboxyl-^{11}C]-nicotinic acid amide

The reaction vessel was then cooled down with liquid nitrogen while it was kept closed with a drying tube filled with P_2O_5, and ca. 5 ml liquid ammonia was poured into the vessel under shaking. Under stirring and warming in a water bath (ca. 30°C) the excess ammonia was finally allowed to distil off under vacuum. The residue was dissolved in 1 ml water and the neutral solution was ready for chromatographic analysis and purification.

Identification and purification

In the first radioactive runs the described synthesis was carried out with CO_2-carrier and about 15 ml of 3-pyridyl lithium solution. After radiochemical assay the aqueous solution was purified (see below) chromatographically, evaporated and the successfully performed synthesis of nicotinic acid amide was demonstrated by IR-spectroscopy.

The radiochemical quality control was carried out routinely by high pressure liquid chromatography (HPLC) using a Varian Micropak Si-5 column (column I) at a Hewlett Packard High Pressure Liquid Chromatograph 1010A. The purification of the total solution

was achieved by simple low pressure liquid chromatography on a prepacked column "Merck-Fertigsäule" (column II) attached to a ProMinent Electronic pump (Chemie und Filter GmbH, Heidelberg, FRG). Details of the separations are given in Table I and II.

TABLE I : Conditions of the HPLC used for the separation of nicotinic acid and nicotinic acid amide

	Column I	Column II
application	analytical	preparative
column	Varian Micropak Si5	Merck Fertigsäule A Kieselgel 60
length	25 cm	20 cm
i.d.	0.22 cm	1 cm
eluent	H_2O	H_2O
flow	0.5 ml/min	4.0 ml/min
inj. sample volume	10 µl	10 µl up to 2 ml

TABLE II : HPLC-separations of nicotinic acid and nicotinic acid amide

Substrate	Column	HETP [mm]	k'	Retention [min]
nicotinic acid	I	1.0	1.9	2.3
nicotinic acid amide	I	0.6	3.2	3.8
nicotinic acid	II	13.3	1.5	2.5
nicotinic acid amide	II	2.1	8.0	9.0

The radioactivity was continuously measured by means of a NaI(Tl)-scintillation crystal, simultaneously with the mass which was monitored with a 254 nm WATERS-UV-Detector [cf. also cit. 3]. Within the detection limit of 10^{-8} m mole nicotinic acid amide, no non-radioactive isotopic amount of the product could be seen. Since the compound is eluted with water no further purification, i. e. desalting is necessary before in vivo application and the volume of the eluted peak (about 16 ml) is quickly reduced to about 1 ml by vacuum evaporation.

RESULTS

The radiochemical yields of ^{11}C-labelled nicotinic acid amide are between 20% and 30%, in the best runs 45% are obtained. The radiochemical purity after chromatographic separation is > 99.9%. Within 60 min [carboxyl-^{11}C]-nicotinic acid amide is ready for delivery in aqueous solution.

With a $^{11}CO_2$ yield of about 1.2 mCi/μA min from a 20 min irradiation at 20 μA the ^{11}C-activity obtained in the product at the time of delivery is about 3 mCi.

ACKNOWLEDGEMENT

The authors wish to thank Prof. G. Stöcklin for his encouragement and helpful discussions throughout this project.

Thanks are also due to the team of the Compact Cyclotron CV 28 for carrying out the irradiations, and to Mr. P. Laufer for his analytical assistance.

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