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CARBON-11, NITROGEN-13 AND OXYGEN-15 : LABELLED MOLECULES

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ABSTRACT

The development of positron emission tomography (PET) has entailed the preparation of radiopharmaceuticals labelled with β^+emitting isotopes such as ^{11}C ^{13}N and ^{15}O.

The use of these isotopes to label molecules for biological applications has led to the development of specific synthesis methods.

After considerations on the general characteristics of ^{11}C ^{13}N ^{15}O, on the choice of molecule to be labelled and on the labelling plan, some examples of labelled molecules are given : ^{15}O - gas and ^{15}O-butanol, ^{11}C-amino-acids and different ^{11}C-ligands for receptors studies, enzymatic labelling with ^{13}N.

CARBON-11, NITROGEN-13 AND OXYGEN-15 - LABELLED MOLECULES

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INTRODUCTION

The development of positron emission tomography (PET), which gives an image of the distribution of a labelled tracer in a transverse section of the body, has entailed the preparation of radiopharmaceuticals labelled with β+ emitting isotopes such as ^{11}C, ^{13}N and ^{15}O.

The use of these radioisotopes to label molecules for biological applications has led to the development of specific synthesis methods suited to the characteristics of the nuclides themselves : short half-life, small mass and high radioactivity required.

GENERAL CHARACTERISTICS OF ^{11}C, ^{13}N AND ^{15}O

These radioisotopes are produced by particle accelerators, usually cyclotrons, and decay by emission of positrons which on contact with matter are annihilated giving off two 511 keV γ rays at 180°.

Their short half-life, while an advantage in that the radiation dose received by the patient is relatively small, confines their place of use to the production site itself.

These products, resulting from nuclear transmutation and being so short-lived, may be obtained with a high specific activity (table 1), even if the theoretical value cannot actually be reached because of contamination by stable elements.

Nuclide	Half-life	Nuclear reaction most commonly used	Theoretical specific radioactivity (Ci/μMole)
Carbon-11	20.4 mn	^{14}N (p, α) ^{11}C	$9.22.10^3$
Nitrogen-13	9.96 mn	^{12}C (d, n) ^{13}N	$1.89.10^4$
Oxygen-15	2.05 mn	^{14}N (d, n) ^{15}O	$9.18.10^4$

Table 1

They may consequently be used to label molecules of some toxicity or present in very small quantities in the body.

CHOICE OF MOLECULE TO BE LABELLED AND LABELLING PLAN

The labelling of a new molecule with a short-lived isotope may require some weeks or months according to the difficulty of the synthesis considered, which means that the molecule must be chosen with special care. This choice relies on the collaboration of biologists, physiologists, physicians, chemists... aware that the process which is to be measured must last no longer than three or four times the half-life of the radioelement.

Moreover the tracer must have a high enough uptake rate inside the organ studied, compared with the injected dose (the total radioactivity injected being limited by the dose delivered to the patient), for the number of disintegrations recorded on the organ to give a statistically significant result.

Once the molecule has been chosen the chemist must plan the labelling procedure.

The synthesis with purification must last no longer than 2 to 2.5 times the half-life of the radioelement, i.e. 40 to 50 minutes for Carbon-11, which means optimising the reaction speeds and finding rapid purification methods.

In addition the synthesis, which will need to be repeated several times a week over some months for injection to patients, must be suited to automation in shielded cells.

The position where the radioelement will be incorporated in the molecule must be examined attentively.

Let us take the example of prazosin, a molecule with a strong affinity for peripheral α_1 receptors. The chemist can introduce a carbon-11 atom in one of two positions : one of the methoxy groups CH_3O- or the - C - group (fig. 1) :

O

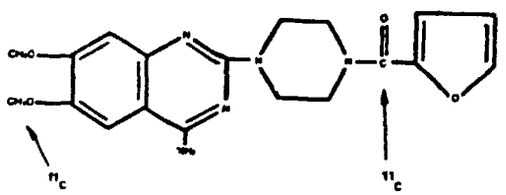

Fig. 1 - Possible positions for labelling in Prazosin

However a study of the pharmacokinetics of this molecule in man has shown that O-demethylation occurs more quickly than cut on the amide. It is obviously better therefore to contemplate labelling on the - CO - rather than on the methoxy groups.

These general remarks will now be followed by a few examples of ^{11}C, ^{13}N and ^{15}O-labelled molecules. Let us start with oxygen-15 which, given its half-life, is suitable only for very simple synthesis.

LABELLING WITH OXYGEN-15

Most ^{15}O-labelled molecules are produced on line :
$|^{15}O| - O_2$, $|^{15}O| - CO_2$, $|^{15}O| - CO$, $|^{15}O| - H_2O$. These molecules are purified by various types of oven or trap set into the circuit (1) (fig. 2 and 3).

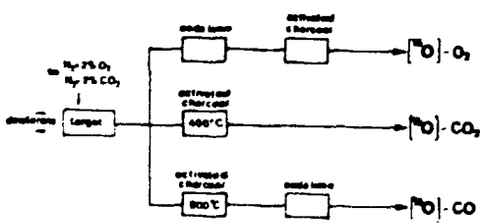

Fig. 2 - On line production of ^{15}O-labelled molecules

Nitrogen protoxide $|^{15}O| - N_2O$ has been obtained by catalytic oxidation of ammonia (2) (figure 4).

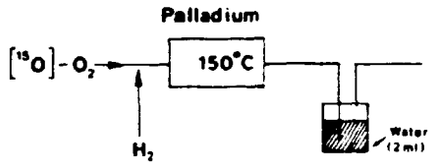

Fig. 3 - Production of ^{15}O-labelled water

$$4\ NH_3 + 4\ {}^{15}O_2 \xrightarrow[300°C]{Pt} 2\ N_2\,{}^{15}O + 6\ H_2O$$

Fig. 4 - ^{15}O-H_2O production from ^{15}O-O_2

More recently $|^{15}O|$ - butanol was synthesized by reaction of oxygen-15 on tributylborane in tetrahydrofurane (3).

$$|CH_3-(CH_2)_3|_3\ B + |^{15}O|-O_2 \qquad |^{15}O|-CH_3-(CH_2)_3-OB$$

After labelling, the butanol is separated out by HPLC in two minutes. Several tens of mCi of $|^{15}O|$ - butanol have been prepared in this way.

LABELLING WITH CARBON-11

Carbon-11 is incontestably the + emitter most widely used to label molecules for medical applications (4). Before its incorporation in the structure of a molecule, the carbon 11 must be obtained in a highly reactive form known as labelled precursor. The most commonly used of these are listed in table 2.

Carbon-11 is generally recovered from the target in one of two forms : $|^{11}C|$-CO_2 and $|^{11}C|$-CH_4. From these two simple molecules it is possible in less than five minutes to prepare more complex but also much more reactive molecules with which an organic synthesis may be undertaken.

It is most important at this stage to keep the high specific activity of the $|^{11}C|$-CO_2 and $|^{11}C|$-CH_4 produced in the target, in other words to avoid as far as possible any isotopic dilution with stable carbon. A labelled molecule will only be a good tracer in fact if the system to be measured is not perturbed, which means that its specific activity must be high.

Very many radiopharmaceuticals have been labelled over the last fifteen years for metabolic or pharmacokinetic research, or to measure certain physiological parameters (flow rate, intracellular pH, membrane permeability... etc). A few illustrations only will be given here.

The first synthesis involved the methylation of demethylated molecules by $|^{11}C|$-methyl iodide. Methyl iodide is the most widely used radioactive precursor to date.

Examples are the synthesis of $|^{11}C|$-methyl quinuclidinyl benzylate (5) (fig. 5) or of $|^{11}C|$ methyl RO 15 1788 (6) (fig. 6), ligands for muscarinic receptors and benzodiazepine receptors respectively.

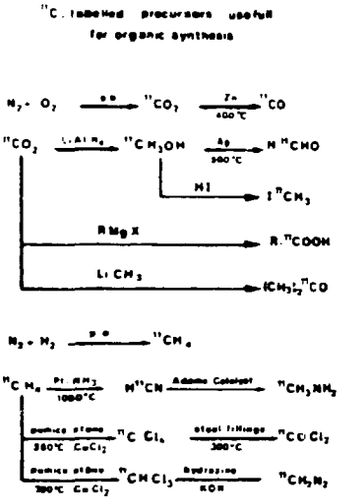

Table 2 - Precursors for ^{11}C-labelling

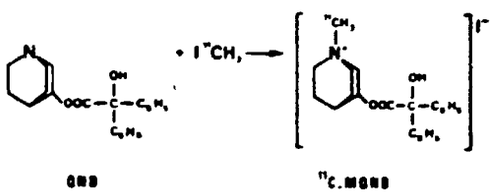

Fig. 5 - [^{11}C]methyl QNB synthesis

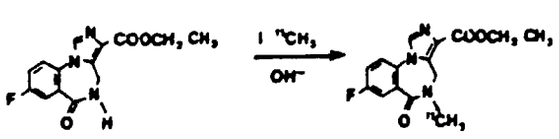

Fig. 6 - [^{11}C]methyl RO 15 1788 synthesis

Another kind of reaction with $|^{11}C|CH_3I$ is the synthesis of an amino acid, methionine (6), much used to study protein synthesis in a given organ (fig. 7).

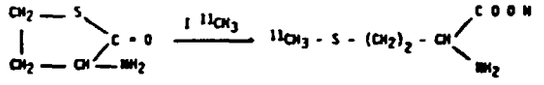

Fig. 7 - $|^{11}C|$methyl methionine synthesis

$|^{11}C|$acetone has been used to label ligand of β-adrenergic receptors : propranolol, practolol, pindolol (8) (fig. 8).

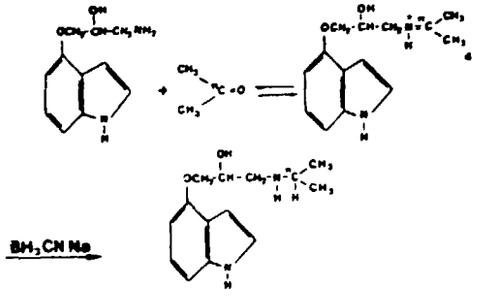

Fig. 8 - $|^{11}C|$ Pindolol synthesis

$|^{11}C|$-phosgene allows a carbonyl function to be inserted between two stereochemically similar amino functions. This has been applied to the labelling of different ligands such as pimozide, ketanserine and more recently CGP 12 177 (9), a β blocker of very high specificity (fig. 9).

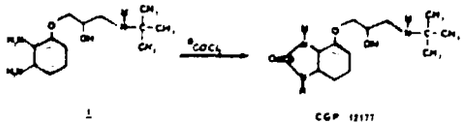

Fig. 9 - $|^{11}C|$ CGP 12 177 synthesis

Many amino acids have been carbon 11-labelled on the carboxyl group by the Bucherer Strecker method, whereby a hydantoïn is prepared by action of KCN on an aldehyde or a ketone then hydrolysed to the corresponding amino acid (fig. 10).

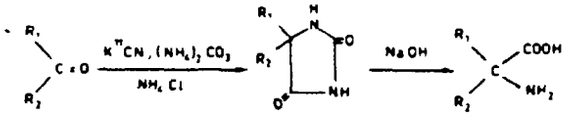

Fig. 10 - Bucherer strecker synthesis

Leucine, valine, tryptophane, phenylalanine have been prepared in this way.

Another more recently reported method involves the action of $H^{11}CN$ on an amino sulphite to give an aminonitrile which is hydrolysed (10) (fig. 11).

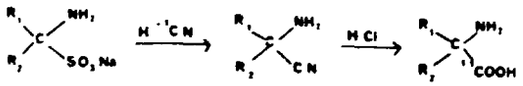

Fig. 11 - Amino acid synthesis from aminosulfite

Many other molecules belonging to the sugar, fatty acid and steroid groups have also been synthesized with ^{11}C.

LABELLING WITH NITROGEN-13

Nitrogen-13 has been used for labelling, though its use is somewhat limited by its 10-minute half-life. However some enzymatic synthesis and one or two organic synthesis have been carried out. ^{13}N may be obtained chiefly in two forms suitable for the purpose : $[^{13}N]$ NH_3 and $[^{13}N]NO_3^-$. ^{13}N-ammonia has been incorporated in many amino acids (11) : glutamic acid, alanine, leucine, valine, aspartic acid...

The general reaction involves the formation of a $[^{13}N]$-L amino acid from $[^{13}N]$-NH_3 and an α keto acid (fig. 12).

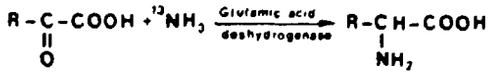

Fig. 12 - Enzymatic synthesis of L amino acid

Certain ^{13}N-amides may also be obtained (fig. 13).

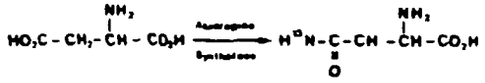

Fig. 13 - Enzymatic synthesis of $|^{13}N|$ amide-asparagine

Of the organic synthesis carried out with ^{13}N (less than ten compounds altogether) one of the latest is $|^{13}N|$phenethylamine (12) (fig. 14).

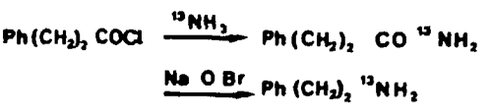

Fig. 14 - Synthesis of $|^{13}N|$phenethylamine

$^{13}NH_3$ reacts on phenylproprionyl chloride for 30 seconds then the $|^{13}N|$ phenylpropricnamide undergoes a 5-minute reaction with sodium hypobromite.

GENERAL CONSIDERATIONS ON ^{11}C AND ^{13}N LABELLING

Once the molecules are labelled, an absolutely pure product ready for human injection, must be obtained. The best method of purifying such molecules is high-performance liquid chromatography on an analytical or semi-preparative scale. The separations take less than 10 minutes and the specific activity of the product may be inferred from the resulting chromatogram. The chromatographic solvents must be highly volatile in order to evaporate very quickly after separation.

Separations of products in two forms D and L have been performed by "Chiral" chromatography. Enzymatic resolutions have also been achieved.

In the case of enzymatic labellings the L form is obtained directly. The increasingly frequent use of enzymes fixed on a solid support reduces the risk of pyrogenicity.

A problem inherent in this type of synthesis is that, because of the short halflife of the radioisotope used it is essential to begin the operation with very high activities (1 to 1.5 Ci in the case of carbon-11 for example). This means that the work must be carried out in shielded cells by means of automatic or at least remoted control systems.

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