ORGANIC RADIOPHARMACEUTICALS LABELED WITH ISOTOPES OF SHORT HALF-LIFE I: ^{11}C-1-DOPAMINE HYDROCHLORIDE

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The preparation of isotopically labeled pharmaceutical and diagnostic agents has seen an almost exponential growth in recent years. The majority of labeled materials available are inorganic compounds or high-molecular-weight organic substances containing a chemisorbed or otherwise bound radioactive isotope. Essentially all of the commercially available isotopes have half-lives of 6 hr or more.

It is our intention to embark on a program of preparation of radiopharmaceuticals involving the incorporation of isotopes of short half-life (which for want of a better definition will involve isotopes of half-life $<$ 6 hr) in organic compounds of known structure. As capabilities for "in house" preparation of short-lived isotopes increase, compound availability will increase. Initially, at least, many organic compounds of interest will also be produced "in house." The techniques required for the preparation of these compounds involve skills not found in any one discipline. Thus the physicist, chemist, biologist and physician must embark on an interdisciplinary approach to the problem.

Our approach to the preparative aspects of such compounds is given in outline form in Fig. 1. There are numerous ramifications to the scheme presented in this figure. In particular we wish to focus attention on the use of a combination of recoil techniques and synthetic techniques used in the preparation of ^{11}C-1-dopamine hydrochloride.

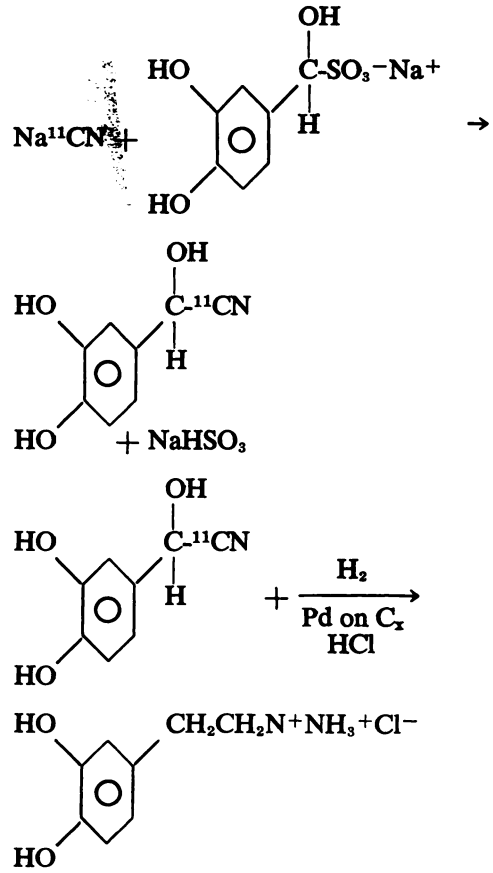
There is nothing new in the concept of incorporating ^{11}C in organic compounds (*1*). Indeed the use of such reactions as the carbonation of a Grignard reagent or the addition of cyanide to a carbonyl group in the preparation of ^{11}C-labeled compounds were pioneered many years ago (*2–4*). Since that time a great deal has been learned about tracer methodology. In addition, much research has been done in hot-atom chemistry in which the knowledge gained bears directly on the problems of preparation of organic radiopharmaceuticals containing isotopes of short half-life. The role of the chemist in such work has been recently reviewed by Welch (*5*). The general field of recoil synthesis has been reviewed by Wolf (*6*) as have the mechanisms of carbon-atom recoil reactions (*7*).

The present work describes a combination of recoil and organic synthesis in the preparation of labeled dopamine. Carbon-11-labeled sodium cyanide was prepared by recoil synthesis (*8*) and used directly in a reaction with the bisulfite addition product of 3,4-dihydroxybenzaldehyde. The cyanohydrin was reduced catalytically, and the amine isolated and purified as its hydrochloride. The reaction sequence is given below.

$$NaCN + p \rightarrow [^{11}C]^{\ddagger} + \text{Fragments}$$

$$^{12}C(p,pn)^{11}C \text{ and } ^{14}N(p,\alpha)^{11}C$$

$$[^{11}C]^{\ddagger} + NaCN \rightarrow Na^{11}CN + {}^{12}C$$



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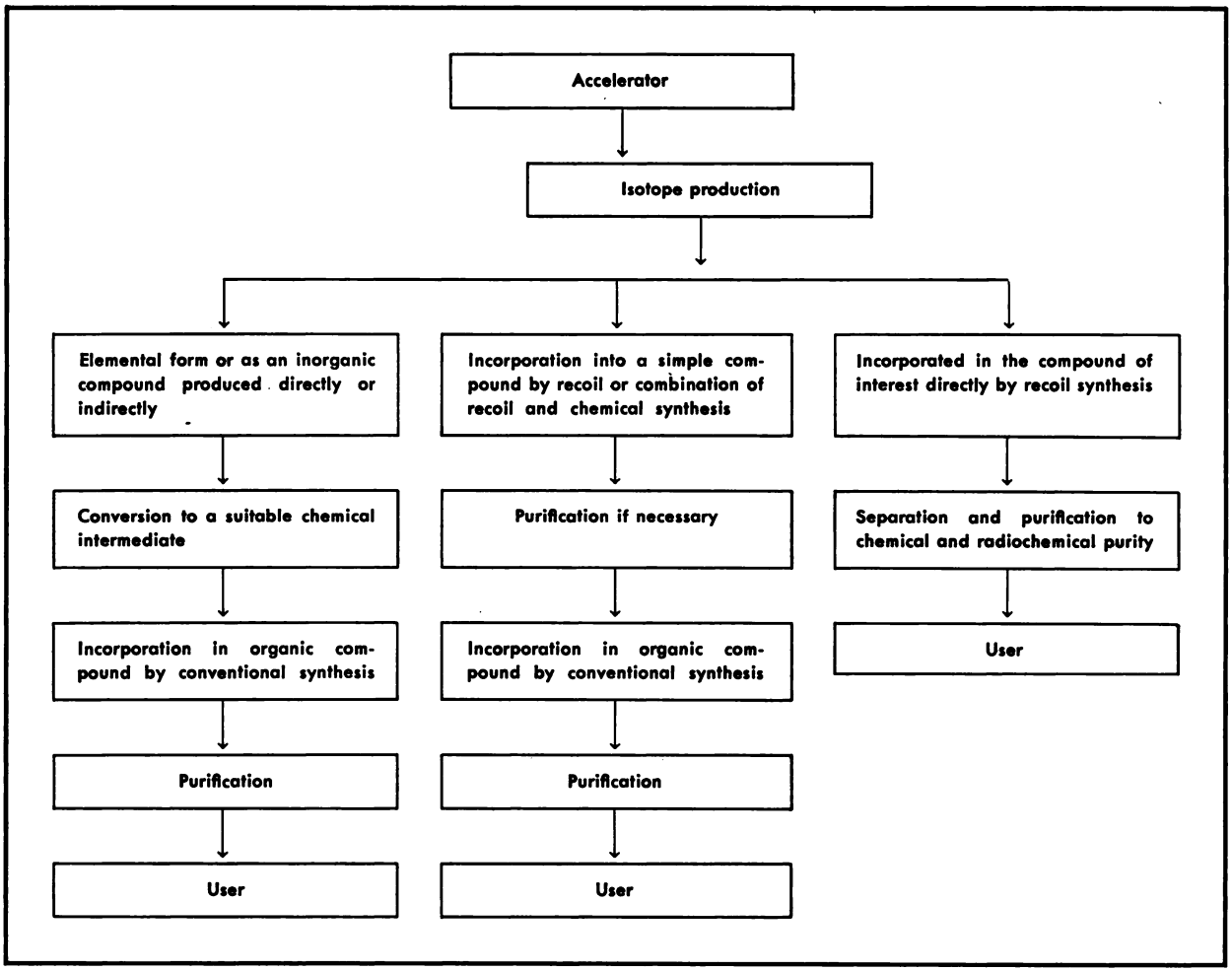

FIG. 1. Organic radiopharmaceutical production.

EXPERIMENTAL

Preparation of carbon-11 cyanide. The direct preparation of recoil labeled cyanide must be adjusted to the proton beam condition in each individual case, but it will be noted that numerous indirect methods can be considered and are currently being investigated, vide infra. The procedure involving the BNL 60-in. cyclotron is as follows. Powdered NaCN (Baker Analyzed Reagent) is wrapped in one thickness of aluminum foil (Wearever Household) in such a way that the NaCN is contained in a volume of uniform thickness and such that its area is the same as the beam area (about 0.45 in.^{2} in this case). The package is cooled by insertion into a milled well in a water-cooled, aluminum target block. (The NaCN can also be melted into the well if desired. The depression is then covered by a thin foil window.) The target is irradiated with 33-MeV protons at current levels between 5 and 10 μA for 30 min. (10 μA is the present upper current level of the BNL cyclotron at the available target area.)

The target block and foil package is then transferred to a lead pig using tongs. When ready for use, the foil package is removed from the pig, cut in half and the dark grey powder is added directly to the pressure reaction bottle (vide infra) via a powder funnel. Normally the hands receive about 10 mR during this particular type of run. Such exposures can be eliminated by remote handling.

A typical irradiation involved a 0.5-gm sample of NaCN. The specific activity of the sodium cyanide at t_0 (t_0 = time at end of cyclotron run) was usually around 480 μCi/mgC (or 118 μCi/mg NaCN). This value clearly depends on the irradiation conditions.

Preparation of ^{11}C-dopamine hydrochloride-^{11}C; [2(3,4-dihydroxyphenyl)-ethylaminehydrochloride-1-^{11}C]. A solution of the bisulfite addition product of 3,4-dihydroxybenzaldehyde (Aldrich Chemical Co., technical grade, purified by sublimation and recrystallization) was prepared by dissolving 0.89 gm (8.55 m*M*) of sodium bisulfite in 5 ml of water and adding 1.0 gm (7.25 m*M*) of the aldehyde. The mixture was stirred until solution was complete. The solution was added to a 500-ml pressure bottle (A. H.

Thomas). ^{11}C-sodium cyanide (0.4 gm; 8.15 m*M*) was added to the bottle along with 40 ml of USP ether. The bottle was sealed and mechanically shaken for 20 min. The two-phase mixture was then transferred to a separatory funnel, the aqueous phase discarded and the ether layer passed through a 6.5-cm i.d. coarse frit, sintered glass funnel containing calcium chloride into a 200-ml round-bottom flask. The ether was quickly evaporated by a rotary evaporator, the flask being held in a water bath at 50°C. Care was taken to avoid complete ether removal because the dry cyanohydrin decomposes rapidly at 50°C. Four to 6 cc of ether can be left in the flask. The contents of the flask were washed into a 50-ml hydrogenation bottle (*9*) using 8 ml of ethanol. Presaturated 5% palladium-on-carbon catalyst (0.30 gm) in 5 ml of absolute ethanol was contained in the hydrogenation bottle. Conc. HCl (0.74 ml; 24.1 m*M*) was then added, and hydrogenation was accomplished on a Paar low-pressure shaker at 60 lb H_2 pressure. The hydrogenation was stopped when the pressure change (in the bottle and hose connection only) in a 5-min interval was less than 0.5 lb (usual duration 20–35 min). The catalyst was removed by filtration through a 6.5-cm i.d. fine sintered glass frit. The solution was then contained in a 500-ml suction flask equipped with a Teflon-coated stirring bar. Slow addition of 100 ml of anhydrous ether to this filtrate, accompanied by cooling and stirring, resulted in precipitation of the ^{11}C-dopamine hydrochloride. It was collected and dried in a vacuum oven at 60°C. The product had an NMR spectrum (trifluoracetic acid) identical with that of an authentic sample.

Analysis. Calcd for $C_8H_{12}O_2NCl$: C, 50.66; H, 6.39. Found: C, 50.16; H, 6.26.

The melting point 222–230°C, with decomposition, of the product as collected is lower than that of the authentic material (melting point 241–243°C with decomposition). One recrystallization from methanol and diethyl ether raises the melting point to that of the authentic material. The recrystallized material is white and nicely crystalline. Each batch was analyzed for residual cyanide. These assays showed typically less than 50 ppm. The crystalline material can be processed for use as required. Specific activities are usually around 30 μCi/mg compound referred to t_0. Overall chemical yields based on the starting bisulfite addition product were in the range of 10–15%, and the weight of product hydrochloride delivered was in the 100–200 mg range.

RESULTS AND DISCUSSION

The major limiting feature in any synthesis is the time available for preparation of a deliverable product. Clearly questions of chemical and radiochemical purity (radionuclide purity in some instances) arise as do the sterility and apyrogenicity of the product. Another factor relating to the needs of the user is the activity level required.

A set of parameters relating to a typical run is given in Table 1. A number of these deserve further exploration in terms of the general aspects of preparing labeled compounds.

Two factors relating to user demand are the specific activity of the substance at time of delivery and the quantity of substance delivered. The controlling variable is the desired specific activity, and this in turn depends on the specific activity of the labeled precursor. Therefore the central question is: What affects the specific activity of the ^{11}C-cyanide and how can this be controlled? (Indeed this information is germane to any preparation requiring labeled cyanide as a precursor.)

The preparation of ^{11}C-cyanide by recoil synthesis is a complex function of the nature and energy of the bombarding particle. The nuclear processes operative in this case are $^{14}N(p,\alpha)^{11}C$ acting on the nitrogen in the cyanide and $^{12}C(p,pn)^{11}C$ acting on the carbon in the cyanide. The nature of the nuclear reaction does not directly affect the yield but the cross sections at the bombarding energy do. In addition, the radiation damage of the cyanide concomitant with the bombardment will affect the gross yield but will have a different effect on the specific activity. The cyanide produced by this method is not carrier free and hence the maximum specific

TABLE 1. CONDITIONS RELATING TO PREPARATION OF ^{11}C-LABELED DOPAMINE HYDROCHLORIDE

Target	NaCN (0.5 gm)	Specific activity, t_0* ^{11}C-Dopamine-hydrochloride	33 μCi/mg salt
Bombarding particle	33-MeV protons	Specific activity at time of delivery, (t_0 + 63 min)	4 μCi/mg salt
Dose in μA sec	9,286 μA-sec (1,800 sec irr.)	Weight of material delivered	180 mg
^{11}C yield	114 mCi	Residual cyanide in product	< 50 ppm
Activity as Na^{11}CN in % of total ^{11}C activity	52%		

* t_0 is defined as the time the cyclotron irradiation is stopped.

activity attainable is directly related to the mass of the target among other factors. Unfortunately no systematic study concerned with yield versus proton energy, dose absorbed (current × time) during exposure, dose rate (proton current during exposure), radiolytic decomposition or thermal decomposition has been carried out. Such a study is currently under way in our laboratory. Regrettably a factor of 10 higher in current will probably not yield a factor of 10 higher in specific activity; however, it is also clear that the specific activities we have obtained can be materially increased.

In addition to the direct method of ^{11}C-cyanide production, we are investigating three additional methods of cyanide production, all of which can yield essentially "carrier-free" cyanide and thus provide a basis for preparing compounds, e.g. ^{11}C-dopamine, at specific activities orders of magnitude higher than those currently attainable. The methods are (including the direct method):

1. $^{11}CN^-$ from irradiation of CN^-; recoil synthesis; compound is its own carrier;
2. $^{11}CN^-$ from irradiation of N_2-H_2 mixtures; recoil synthesis; $^{11}CN^-$ "carrier free" (*10,11*);
3. $^{11}CN^-$ from irradiation of metal nitrides; recoil synthesis; $^{11}CN^-$ "carrier free" (*12,13*);
4. $^{11}CN^-$ from the reaction of $^{11}CO_2$, K and NH_3; recoil synthesis plus conventional synthesis; $^{11}CN^-$ "carrier free" (*2*)*.

Each of these methods is also being studied with a view towards the optimization of irradiation conditions.

To date there is very little in the literature about the general practice and specifics of labeling organic compounds for medical use with ^{11}C and other isotopes of short half-life. A paper on some aspects of production of ^{11}C compounds for use in nuclear medicine using the photonuclear reaction $^{12}C(\gamma,n)^{11}C$ as a ^{11}C source has been published (*14*). In addition some work on the preparation and use of ^{11}CO has also appeared (*15,16*).

Unfortunately there is also some data in the literature of doubtful validity. Thus the report (*17*) on the chemical results of labeling of glycine and urocanic acid is almost certainly in error, based on what earlier research had demonstrated (cf. data in Ref. *6*). While there is no question that some recoil labeled glycine existed in the matrix of the irradiated bulk glycine, the yield *at best* was no more than 5%. Another report has dealt with the fixation of ^{11}C in the cells of proton-irradiated blood (*18*). Again, from what is known about systems of this sort, the major fraction of the ^{11}C will be present as compounds bearing little or no relation to the normal constituents of blood. This is not to say that the association of these many compounds with the whole blood cannot be useful in research or may not serve some diagnostic purpose, but what is clear is that the normal constituents of blood are labeled at a very low level indeed (*6*).

^{11}C-dopamine hydrochloride as described in this paper can be prepared at activity levels useful for biological and medical work. Considerably higher specific activities are attainable but some research still needs to be done.

Current research efforts in our laboratory are directed toward the exploration of the many facets involved in the preparation of specific organic compounds labeled with isotopes of short half-life. The isotopes of current interest to us include ^{11}C, ^{13}N, ^{15}O, ^{18}F, ^{123}I, other halogens and some of the isotopes of sulfur, selenium, tellurium, phosphorous and iron. Our goals are (1) development of fast synthetic techniques, (2) development of fast separation and purification methods, (3) maximization of specific activity, (4) development of sterility and apyrogenicity criteria valid for these compounds of short half-life and (5) development of simple methods applicable to the preparation of whichever of these products may prove to be useful so that their "in-house" preparation can be realized. Our purpose is to prepare specific organic compounds labeled with isotopes of short half-life which may be of use to medical research and clinical practice.

SUMMARY

A method involving recoil synthesis and conventional synthesis is described for the preparation of ^{11}C-1-dopamine hydrochloride. The parameters of the problem involving the preparation of specific organic compounds labeled with isotopes of short half-life are discussed.

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