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Radioiodination Techniques for Small Organic Molecules

ROBERT H. SEEVERS*[1] and RAYMOND E. COUNSELL

The Interdepartmental Program in Medicinal Chemistry, The University of Michigan, Ann Arbor, Michigan 48109

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I. Introduction

Radioactive isotopes of iodine have proven to be very useful for labeling both large and small molecules. A great many methods have been published for attaching radioiodine to target molecules. The techniques for radioiodinating proteins,[2–8] nucleic acids,[9] and cell surfaces[10] have been reviewed recently. Methods for radioiodinating small organic molecules, only some of which are derived from those developed for larger targets, are reviewed here.

A. Definitions

It will be helpful to define some terms that are used in the discussion of radioiodinated substances.

Carrier is the term used to refer to stable iodine-127 present in or added to the preparation of a radioiodine-labeled compound.

Carrier free was used in the past to refer to a labeled compound in whose preparation no stable iodine was used. Owing to the small molar quantities of radioactive iodine actually used in practice and the relative ubiquity of tiny amounts of stable iodine, this term can be misleading and has generally been replaced by *no carrier added.*

Specific activity is a measure of the radioactivity of a labeled molecule. It is generally expressed in millicuries per millimole or millicuries per milligram of compound (1 mCi = 3.7×10^7 disintegrations/s). Maximum specific activity is obtained when each molecule in the preparation is radiolabeled.

B. Isotopes of Iodine

Natural iodine has a relative atomic mass of 127 and contains 74 neutrons. It is not radioactive. A number of artificial isotopes of iodine that contain more or less than 74 neutrons have been prepared. These isotopes decay by emitting one or more forms of radioactivity. The isotopes of iodine most useful for radiolabeling are ^{131}I, ^{125}I, and ^{123}I. Some of their important properties are listed in Table I.

C. Uses of Radioiodinated Compounds

The great usefulness of radioactively labeled compounds in general stems primarily from the ease of detecting and measuring extremely small amounts of them. Few molecules of interest to chemists and physicians contain iodine, but it has been attached to a great variety of compounds for tracer work. Carbon-14 and tritium (hydrogen-3) are also extensively used as radiolabels. Why have people gone to all the trouble of labeling molecules with iodine that does not belong there, instead of using ^{14}C or ^{3}H which are isotopes of elements that are ubiquitous in organic molecules?

The isotopes of iodine have two major advantages over ^{14}C and ^{3}H. The first is the type of radiation they emit on decay. ^{14}C and ^{3}H are β emitters, which is to say their decays result in the ejection of an electron with an energy characteristic of the isotope. These energies are listed in Table I. In practice, β particles are detected by the fluorescence they cause in a solution containing a sensitive dye (a scintillation cocktail).

All of the iodine isotopes in normal use are γ emitters. Their decays result in the emission of one or more

Robert H. Seevers, born in 1954, received his B.S. in Chemistry from Lehigh University in 1976, where he first became interested in medicinal chemistry. He obtained his Ph.D. in medicinal chemistry from the University of Michigan in 1981. He is currently working in the Chemistry Division at Argonne National Laboratory. His research interests include steroid biochemistry, CNS pharmacology, radiopharmaceuticals for diagnosis and therapy, and improved synthetic methods for radiolabeling organic molecules.

Raymond E. Counsell was born in 1930. He obtained his B.S. in pharmacy from the University of British Columbia in 1953 and his Ph.D. in medicinal chemistry from the University of Minnesota in 1957. Prior to joining the faculty of The University of Michigan in 1964, he was a Senior Research Chemist with G. D. Searle and Co. for seven years. While his present position is Professor of Pharmacology in the Medical School, he also holds a joint appointment as Professor of Medicinal Chemistry in the College of Pharmacy. His major research interests involve the design and synthesis of chemical regulators of biological processes as well as radiologic agents for diagnosis.

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TABLE I. Properties of Some Important Isotopes

nuclide	half-life	decay mode	particle energy, keV	γ emissions, keV
^{3}H	12.35 y	β^-	18.6	none
^{14}C	5730 y	β^-	156	none
^{123}I	13.2 h	EC		14 γ: 159–784; 159 (99%)
^{125}I	60.14 d	EC		1 γ: 35 (100%)
^{131}I	8.04 d	β^-	606 (90.4%) 333 (6.9%)	19 γ: 80–723; 364 (83%) 637 (7%), 284 (6%), 80 (6%)

photons whose energies are characteristic of the isotope. The γ energies of ^{123}I, ^{125}I, and ^{131}I are listed in Table I. Iodine-131 is also a strong β emitter. The advantage of the iodine isotopes in this respect is that γ rays are detectable directly without the use of a costly and cumbersome scintillation system.

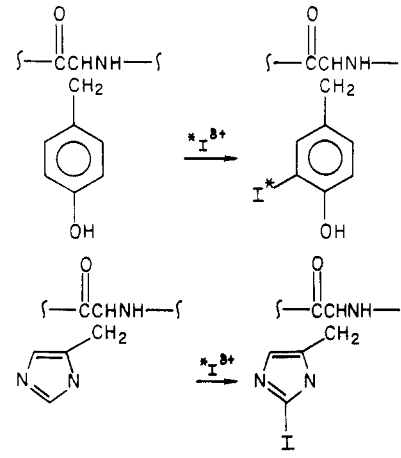
The second advantage of the iodine isotopes is the relative shortness of their half-lives (see Table I). Since the maximum specific activity theoretically attainable with a given isotope is inversely related to its half-life, the use of an isotope with a shorter half-life is desirable. Thus, one atom of ^{131}I substituted into a molecule of insulin produces a disintegration rate 200 times as high as would be obtained if all 263 carbon atoms in the molecule were ^{14}C.[11] A short half-life also reduces the radiation exposure of patients treated with a radioactive isotope. Of course, the short half-life of the iodine isotopes, especially ^{123}I, creates definite synthetic challenges as well.

Radioiodinated compounds have two primary uses. The first is in radioimmunoassay (RIA). This procedure, developed more than 20 years ago by Yalow and Berson,[12] uses antibodies raised against a specific molecule, called an antigen, for its detection in minute amounts.[2,13] The key technique is the competition of a radiolabeled antigen with the unlabeled antigen to be quantitated for binding with the antibody. High specific activity is of great importance.[11,13-17] Iodine-125 is the isotope primarily used for this purpose; its soft γ emission is readily detected and yet easy to protect personnel from. The 60-day half-life of ^{125}I means that labeled materials can be prepared and stored for some time subject to their chemical and radiochemical stability.

Iodine-131 is rarely used for RIA anymore. While its shorter half-life offers the potential of higher specific activity, it has a number of drawbacks. ^{131}I is not usually available without added carrier, and its decays are not counted as efficiently as those of ^{125}I.[11] A shorter half-life also means that labeled compounds must be prepared more often. Finally, the radiation from ^{131}I is much more penetrating, and so requires increased protection for the people involved in the synthesis and use of the radioiodinated compounds.

The other major use of radioiodinated compounds is in nuclear medical imaging. Here compounds that localize in a particular organ or tissue are used to carry a radiolabel whose emissions are detected outside the body. Since the imaging is external, the radioactivity must be strong enough to get out of the body but not so strong that it cannot be detected by the cameras now in use. This requires compounds labeled with γ-emitting isotopes, so carbon-14 and tritium are not useful for this purpose. Carbon-11, a positron emitter, is receiving increasing use in imaging,[18] but its extremely short half-life means that it can only be used in locations with nearby cyclotron isotope production facilities.

SCHEME I. Radioiodination of Proteins on Tyrosyl and Histidyl Residues



The two most important isotopes of iodine for nuclear medicine imaging agents (radiopharmaceuticals), are ^{123}I and ^{131}I. The short half-life and γ energy of iodine-123 make it ideally suited for this use.[19] Iodine-131 with its longer half-life and stronger radiation gives a much larger radiation dose to the patient and is less well suited for imaging with the γ cameras currently in use. The increasing availability of ^{123}I in recent years has enabled it to replace ^{131}I in imaging agents in many instances.[19,20]

Iodine-125 does not have a strong enough γ emission to be detected outside the body. It is extremely useful, however, for preliminary studies in the development of a potential radiopharmaceutical imaging agent. These studies require determination of the tissue distribution of the radioiodinated compound at various time periods in animals. Each animal is killed at a set time after the administration of the labeled compound, and its organs are removed and counted for radioactivity in a γ counter such as that used for RIA. Iodine-125 is ideal because of its longer half-life.

A labeling procedure developed for one isotope of iodine should be adaptable to another, provided the time required by the procedure, the half-lives of the isotopes, and the chemical form in which the radioiodine is supplied are taken into account.

II. Oxidative Radioiodination Techniques

A. Radioiodination of Proteins

A great many radioiodination procedures were originally created for labeling proteins, and it is instructive to begin this discussion by considering the chemistry involved in protein radioiodination. The basic reactions are shown in Scheme I. Some positive iodine species react with the tyrosyl and, to a lesser extent, histidyl residues of the protein.[21,22] Studies on the mechanism of the reaction of iodine with tyrosine and other phenols

TABLE II. Compounds Labeled by Reaction with Molecular Iodine

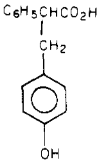
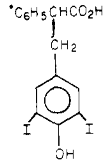
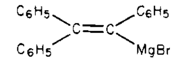
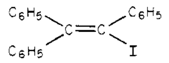
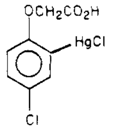
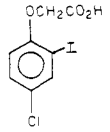
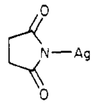
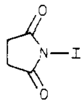
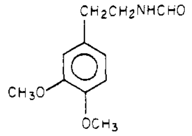
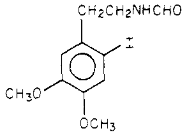
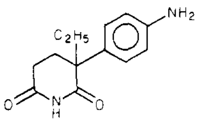
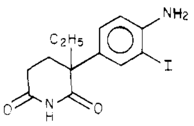
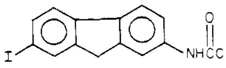
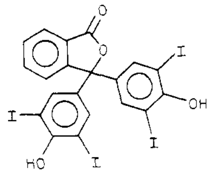
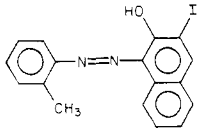
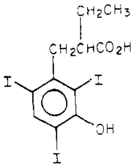
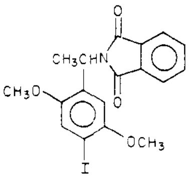
starting material	product	ref
		33
		34
		35
		36
		37
		38

TABLE III Compounds Labeled by Use of Iodine Monochloride

compound	ref
	42
	43
	44
	45
	46, 47
bleomycin (see Figure 1)	48-50

in stoichiometric amounts indicate that it is the phenolate anion which is attacked.[21,23-25] The mechanism of the iodination of histidyl residues is less well studied.

B. Molecular Iodine

In the early days of protein labeling, methods used for iodinating phenols with stable iodine were, quite naturally, applied to radioiodination. Radioactive molecular iodine was the most commonly employed labeling reagent.

Since radioactive iodine is usually available as sodium iodide, some means of oxidation must be employed to obtain radioactive molecular iodine. Pressman and Keighly[26] circumvented this (at the expense of specific activity) by adding sodium iodide-^{131}I to carrier molecular iodine before reacting it with the protein.

Others have used a number of different oxidizing agents to obtain radioactive molecular iodine from iodide. Yalow and Berson[12,27] were able to get it by use of nitrous acid followed by extraction into carbon tetrachloride. Others have used ammonium persulfate,[28] hydrogen peroxide,[29] ferric sulfate,[30] or an iodide/iodate system[31] for oxidation prior to protein radiolabeling. A recent variation is to treat sodium iodide-^{131}I in one vessel with chromic acid and carry the volatile species produced (which may or may not have been molecular iodine) to a separate vessel containing a protein solution by using a stream of air.[32]

There are a number of disadvantages to the use of molecular iodine for radiolabeling: (1) The maximum possible radiochemical yield is 50% because half of the label ends up as radioiodide (which also makes purification more difficult). (2) The prior oxidation step involves some loss of radioactivity and increased radiation exposure to the operator. This can be avoided by purchasing radioactive molecular iodine, which is now commercially available, but which costs much more than labeled sodium iodide. (3) The volatility of molecular iodine greatly magnifies the hazards of the labeling procedure. In spite of these difficulties, radioactive molecular iodine has been used to label small organic molecules. Some examples are shown in Table II.

C. Iodine Monochloride

In 1958 McFarlane[39] published a technique for protein radioiodination that largely avoided the disadvantages mentioned above by the use of iodine monochloride (ICl). The reagent was prepared by treating unlabeled ICl with radioactive sodium iodide. The iodine-chlorine bond in ICl is polarized with a partial positive charge on the iodine, so the radiochemical yield is potentially 100%. Studies on the iodination of phenol and substituted phenols with unlabeled ICl indicate that the mechanism is substantially the same indicated above for molecular iodine: electrophilic attack on the phenoxide ion followed by slow loss of a proton.[40] The electrophilic species has been suggested to be H_2OI^+ at low pH and ICl at higher pH.[40] Another study proposed HOI as the electrophile.[41] The use of ICl for protein radiolabeling has been reviewed recently.[6]

Iodine monochloride has been used to label small organic molecules that possess a site activated to electrophilic attack. Some examples are found in Table III. The iodination of bleomycin with ICl[48-50] is very interesting. This large antibiotic (Figure 1) has an imidazole ring where labeling was expected to take place.[48,49] Eventually, this was shown to be the case by an NMR study, which suggested, however, that the product formed under the radioiodination conditions

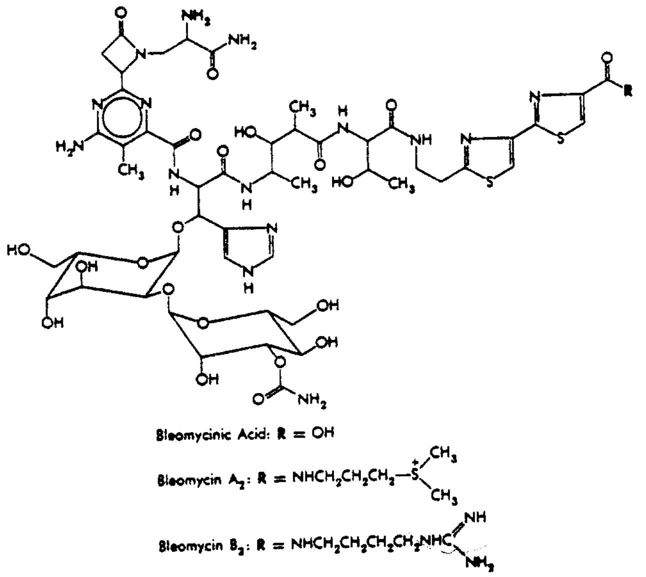


Figure 1.

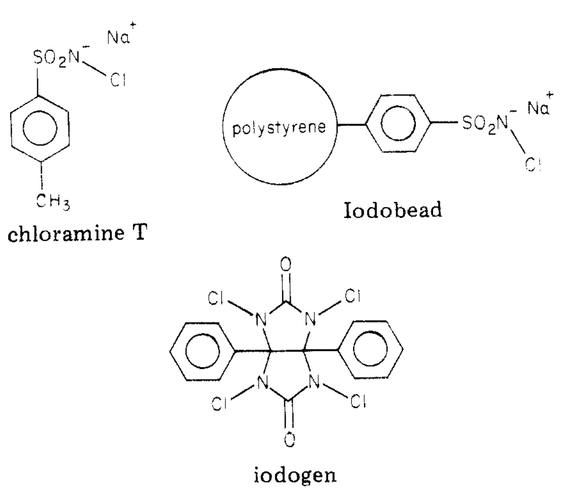


Figure 2.

was the diiodo derivative.[50]

Electrophilic substitution is not the only way in which ICl can be used for radioiodination. Electrophilic addition across a double bond has also proven useful, especially in the labeling of fatty acids and derivatives. Triolein[51,52] and several unsaturated fatty acids[53] have been radioiodinated this way. A variation on this was the use of iodine monobromide to label triolein.[54]

D. Chloramine T

The most widely used radioiodination technique is that of Hunter and Greenwood.[55,56] Their method uses chloramine T (Figure 2) as the oxidizing agent. In aqueous solution it forms HOCl, which is thought to be the actual oxidizing species.[2,57] This reacts with the radioactive iodide present to form some electrophilic iodine species, possibly H_2OI^+.[58] A study of the tritium kinetic isotope effect in the iodination of L-3-iodo[5-^{3}H]tyrosine by molecular iodine or chloramine T and KI at iodine concentrations similar to that used in radioiodination reached two conclusions: the carbon-tritium bond cleavage was the rate-determining step, and the iodinating species was the same in both cases.[59] In fact, sodium hypochlorite[60] and chlorine water[61] have both been used as oxidants for radioiodination. The use of chloramine T for protein radiolabeling has been reviewed recently.[2,3]

TABLE IV. Compounds Labeled by Use of Sodium Iodide and Chloramine T

compound	ref
fluorescein	62
chloroquine	63
N-substituted uracils	64
bleomycin	48, 69
N,*N*-dimethylaniline	70
ω-(4-aminophenyl) fatty acids	71
thyronine analogues	72
uracil	73
cytosine	73
uridine	73
histidine	73
tyrosine	73

One problem with the chloramine T method of radioiodination is that the compound to be labeled is exposed to harsh oxidizing conditions. This causes a number of undesirable side reactions, including chlorination,[62-64] oxidation of thiol and thioether groups,[65,66] and cleavage of tryptophanyl peptide bonds.[67,68]

Despite these difficulties, chloramine T has been used to radioiodinate a number of small organic molecules. These are shown in Table IV. The chloramine T radioiodination of bleomycin[48,69] produces an unstable product, thought to be the result of N–I bond formation,[48] that has poor biological activity.[69] This is in contrast to the result with iodine monochloride.[50] However, bleomycin labeled by the chloramine T method has been used successfully in a radioimmunoassay.[162]

Recently, a solid-phase version of chloramine T, called Iodobeads (Figure 2), has become available.[74] Its usefulness for radioiodination has been demonstrated.[75]

E. Iodogen

An oxidizing agent resembling a 4-fold chloramine T called iodogen (Figure 2) has been compared to chloramine T and iodine monochloride for protein radioiodination and found to give similar or somewhat lower radiochemical yields but with less oxidative damage.[8,66,77-80]

Iodogen is essentially insoluble in water[76] and so can be used as a thin film coating the wall of the radioiodination vessel, thus permitting labeling with very little contact of the organic compound with the oxidizing agent.[8,66] Iodogen has been used in the radioiodination of a series of uracils[64] and a series of thiouracils.[81] In the latter case iodogen gave better results than chloramine T.

F. Electrolytic Methods

It is possible to use an electrolytic cell to oxidize iodide to iodine. This has been applied to radioiodination of proteins.[82-85] The radioactive iodide which is produced by the reaction can be reoxidized, so the potential radiochemical yield is 100%. This method has the advantage of not exposing the material to be radioiodinated to harsh oxidizing conditions, but requires some special equipment and expertise.

Compounds radioiodinated by the electrolytic technique include a nitrogen mustard derivative of estradiol 3,17-bis(phosphate),[86] diethylstilbestrol bis(phos-

TABLE V. Compounds Labeled by Enzymatic Radioiodination

compound	ref
phenol	92
fluoresceinamine	95
estradiol	92, 96
estrone	96
tyrosine	92, 97
uracil	73
uridine	73
cytosine	73
histidine	73
phospholipids	98

phate),[86] and estradiol.[87] For the first two, the structure of the products is unknown.[86] Electrolytic labeling of estradiol produced the 2-iodo, 4-iodo and 2,4-diiodo derivatives in 20%, 23%, and 4% yields, respectively.[87]

G. Enzymatic Methods

There are enzymes called peroxidases that, in the presence of hydrogen peroxide, iodinate tyrosines. It is one such enzyme that iodinates thyroid peptides and proteins in the body. Peroxidase enzymes, in particular lactoperoxidase, have been put to use for radioiodination.[88,89] This technique has been simplified by use of immobilized forms of the enzyme.[90-92] Histidines can also be radioiodinated by lactoperoxidase but at a much slower rate than tyrosines.[93,94] Protein radioiodination with lactoperoxidase has been reviewed recently.[4]

Enzymatic radioiodination is a milder alternative to techniques that use chemical oxidants. This is especially true when the source of hydrogen peroxide is another enzyme system such as glucose oxidase.[4] Several compounds that have been labeled enzymatically are listed in Table V. Interestingly, two different attempts to use lactoperoxidase to radioiodinate bleomycin resulted in labeling the enzyme instead.[48,69] Apparently, the imidazole ring in bleomycin is not as easy to radioiodinate as lactoperoxidase itself.

Lactoperoxidase has been used to radioiodinate phospholipids.[98] Most labeling occurs on the fatty acid attached to C-2 of glycerol. Since these fatty acids tend to be unsaturated, one would expect that an addition reaction has occurred. However, dipalmitoylphosphatidylcholine (which has no carbon–carbon double bonds) is also readily radioiodinated by lactoperoxidase,[98] but the radiolabeled product formed in this case was not characterized.

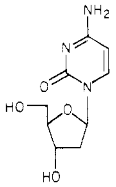
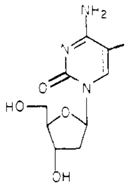
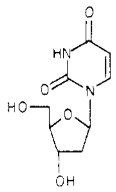
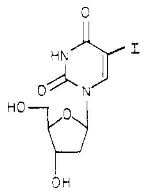
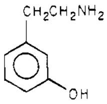
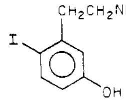
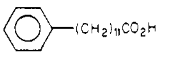
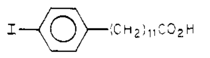
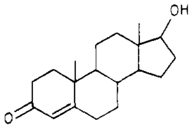
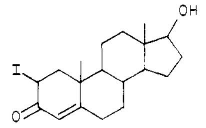
H. Other Direct Oxidation Methods

Other methods of radioiodination involving direct oxidation of labeled sodium iodide in situ with various oxidizing agents have been used. Several examples are found in Table VI. All of the oxidizing agents (H_2O, HONO, HNO_3, etc.) are capable of oxidizing iodide to iodine and then reoxidizing the iodide formed by the iodination reaction. The theoretical radiochemical yield is therefore 100%.

I. Prosthetic Groups

Many small organic molecules do not have activated aromatic groups that lend themselves to direct radioiodination using chloramine T or another oxidant. In addition, these compounds may not be stable to harsh oxidizing conditions. One way to circumvent these problems is the use of prosthetic groups.

TABLE VI. Compounds Labeled by Various Direct Oxidation Methods

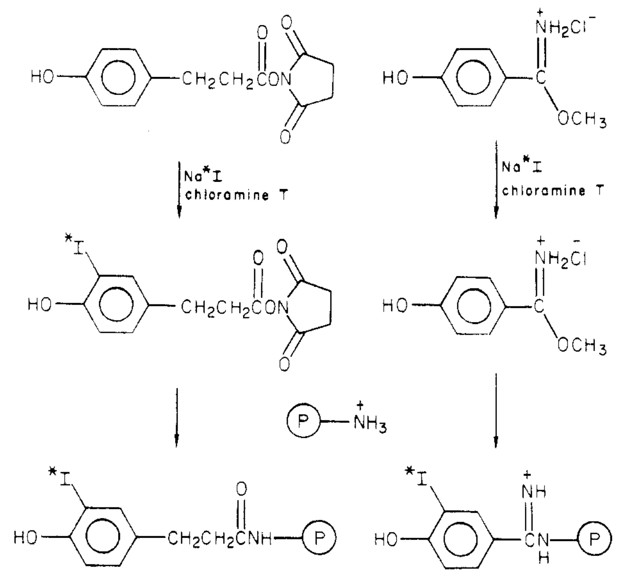
starting material	oxidant	product	ref
	Cl_2/H_2O		99
	HIO_3		100
	HNO_3		101
	H_2O_2		102
	H_2O_2		103, 104
	H_2O_2		105

A prosthetic group for radioiodination contains some type of activated aromatic moiety to receive the label and some connecting bridge to covalently attach it to the molecule to be labeled. It is possible to label the prosthesis and then couple it to the target molecule in two separate reactions, thereby avoiding exposure of sensitive functionalities in the target molecule to oxidation. The coupling reaction must be efficient to avoid wasting the label. The alternative is to couple the prosthesis to the target molecule and then do the labeling. The presence of the active aromatic group in the prosthesis then permits the use of milder radioiodinating conditions. In general, if the extra baggage of the prosthetic group and connecting bridge do not interfere with a molecule's binding properties, then the radioiodine label will give an accurate picture of the behavior of the parent compound.

An early example of the use of prosthetic groups for radioiodination was the treatment of insulin with 4-[^{131}I]iodobenzenediazonium chloride.[106] Presumably, azo linkages were formed with free amino groups in the protein. A similar reagent in use today is 3-[^{125}I]iodo-4-aminobenzenesulfonic acid, which is diazotized and then used to label cell surface proteins.[10,107]

Bolton and Hunter[108] developed an acylating agent for radioiodinating proteins: *N*-succinimidyl 3-(4-hydroxyphenyl)propanoate. The prosthesis is first labeled by the chloramine T method and then coupled to the protein (Scheme II). A related reagent is methyl 4-hydroxybenzimidate hydrochloride, which can be used to label proteins in a similar manner[109,110] (Scheme II). It has the possible advantage of maintaining a positively charged group in that position of the labeled molecule. A third such reagent is *tert*-butyloxycarbonyl-L-tyrosine *N*-hydroxysuccinimide ester.[111] The preparation and

SCHEME II. Radioiodination of Proteins via Prosthetic Groups (P = Protein)



use of the Bolton–Hunter and related reagents for protein labeling have been reviewed.[5,7]

Prosthetic groups have been used to radioiodinate a number of small organic molecules. The primary purpose has been to provide ^{125}I-labeled antigens for radioimmunoassay.[11] There are three major prosthetic groups in use: tyrosine methyl ester (TME), tyramine (TA), and histamine (HA). These are usually attached to the target molecule by an amide linkage, either through an existing carboxyl group or by a carboxyl added by chemical modification. Two common bridges are the hemisuccinate formed by reaction of succinic anhydride with an existing hydroxyl and the (carboxymethyl)oxime obtained by reaction of (carboxymethyl)hydroxylamine with an existing ketone. These types of chemical modifications are depicted in Scheme III. Two isomers (syn and anti) are possible with the (carboxymethyl)oximes. In the case of norgestrel (carboxymethyl)oxime, these isomers were separated and the labeled TME derivatives were found to have identical immunoreactivity.[130] Radioiodination, usually by the chloramine T method, can produce both the mono and diiodo derivatives.[121,123,135,163] Table VII lists compounds with the bridges and prosthetic groups used to label them.

A serious problem, referred to as bridge binding, has been noted in the use of compounds labeled by means of prosthetic groups for RIA.[14,122,162] It occurs when the same bridge is used for two purposes. A bridge is needed to attach the prosthetic group to be labeled. A bridge is also required to conjugate the compound to a protein for the purpose of raising antibodies, since most organic molecules are too small to be antigenic by themselves. Antibodies raised in this way may recognize the bridge as well as the compound itself. The result, if the same bridge is used in the attachment of the prosthetic group, is that the antibodies bind the labeled compound with a much greater affinity than the unlabeled compound one wishes to quantitate. Since it then requires a greater amount of the unlabeled compound to displace the same amount of labeled compound from the antibody, the net result is a decrease in the sensitivity of the assay. This problem can be prevented by using one sort of bridge for protein conjugation and another for attachment of the prosthetic group to be radiolabeled.[115,122,124,125,132]

The use of a prosthetic group will not give good results if the binding properties of the parent compound are disturbed. This was the case in the use of the Bolton–Hunter reagent[108] to label bleomycin:[69] the radioiodinated product had no biological activity. Similarly, the use of bovine serum albumin coupled to estradiol as a target for radioiodination gave a product devoid of immunoreactivity,[114] possibly because the aromatic ring of estradiol was iodinated instead of the protein.

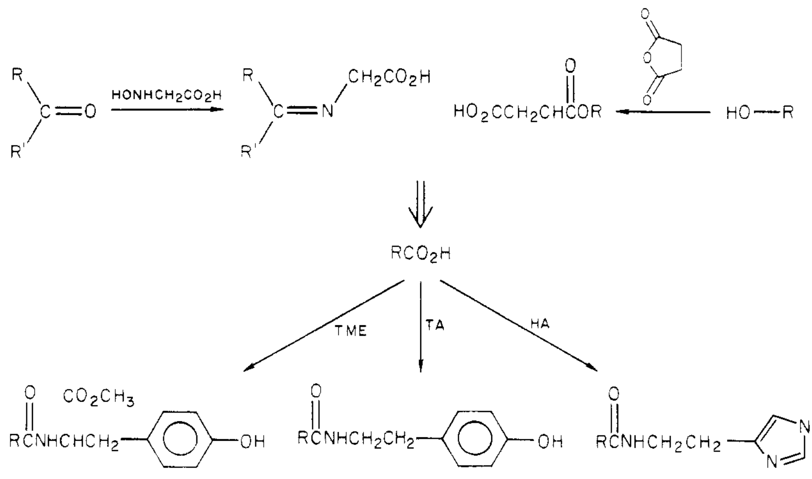
There are cases where the retention of biological properties is not crucial to the detection of certain compounds. A venerable example is the use of 3-[^{131}I]iodopyridinium hydrazonylacetate to determine keto steroids.[164] Another more recent example is the use of 3-[^{125}I]iodo-4-hydroxyphenylpropionyl carbohydrazide in the detection of periodate oxidized nucleoside derivatives.[165]

III. Substitution Radioiodination Techniques

A. Exchange in Solvent

The least complex means of radioiodination of small organic molecules is the substitution of radioactive iodide for a stable iodine atom already incorporated in

SCHEME III. Attachment of Prosthetic Groups



the molecule. This can often be done simply by heating the compound with radioactive iodide in an appropriate solvent such as water or acetone. The solvent must be able to dissolve both the organic molecule and the inorganic iodide.

The iodide exchange labeling of iodo aromatics does not proceed by the straightforward nucleophilic aromatic substitution process one might expect because of the extremely small quantities (approximately 10^{-9} mol) and, hence, low concentrations of radioactive iodide used. Instead, a heterogeneous mechanism dominates.[166,167]

Since most of the iodine atoms in the final product will be ^{127}I, it is not possible to prepare compounds of very high specific activity by an iodine-exchange reaction. Nevertheless, the modest specific activities obtainable by this method are satisfactory for a number of purposes such as some kinds of radiopharmaceuticals. Table VIII contains examples of compounds prepared by this method.

Some compounds exchange a great deal more readily than others. The rate of exchange of a series of nicotinic acid derivatives was increased by electron-withdrawing substituents.[179] In the same series, transformation to the *N*-oxides greatly enhanced the exchange rate.[179]

It has recently been observed by ourselves and others that acetic acid is a very useful solvent for exchange of iodinated aromatics.[182,183]

B. Exchange in Melt

Many compounds do not exchange well with radioactive sodium iodide under reflux conditions in a solvent. An alternative to exchange in solvent is exchange in melt. There are three basic variations of the melt exchange.

The simplest form of melt exchange is where the reaction takes place in a melt of the nonradioactive aromatic compound itself. The compound to be labeled must be stable at its melting point and have a high enough dielectric constant to dissolve the radioiodide. This technique was first developed for the exchange labeling of *m*-iodohippuric acid[184] and has been used for labeling of *o*-iodohippuric acid[185,186] and 2,3,5-triiodobenzoic acid.[187] In our laboratory, we have used this method to label cholesteryl 4-iodobenzoate and cholesteryl 2-(3-iodophenyl)acetate; the radiochemical yields were rather low for these compounds.[188]

A variation of this technique was used to label 4-iodophenylalanine and 5- and 6-iodotryptophan by exchange in the melt of low-melting acyl derivatives with the decay product of ^{123}Xe.[189] This can be thought of as a combination of the melt method and recoil labeling (vide infra).

One way to avoid the difficulties of compounds with low dielectric constants is to do the exchange in a melt of acetamide.[190] Acetamide melts at 82 °C and is stable above 200 °C; the reaction is run at about 180 °C. All three isomeric iodobenzoic acids exchange well under these conditions.[190] The polyiodinated X-ray contrast media 3,5-diacetamido-2,4,6-triiodobenzoic acid (diatrizoic acid) and 5-acetamido-2,4,6-triiodo-*N*-methylisophthalamic acid (iothalamic acid) were also labeled in this way.[191] In our study of iodinated aromatic cholesteryl esters,[188] we used the acetamide melt method to label a series of compounds that included the 2,3,5-triiodobenzoate, 3,4,5-triiodobenzoate, 2- and 3-iodobenzoate, 2- and 4-iodophenylacetate, 4-iodophenylpropanoate, and the 4-iodophenylbutanoate. The radiochemical yields for the various esters were modest to good.[188] Similarly, we have labeled a triglyceride analogue, 1,2,3-trihydroxypropane 1,3-dipalmitate 2-(3-iodobenzoate), by the acetamide melt procedure.[192] Work done on exchange in a melt of formamide indicates that radioiodine exchange works well for aromatics with electron-withdrawing groups and poorly for those with electron-donating groups.[193]

The third type of melt procedure is actually done at a temperature below the melting point. The substrate is heated in ammonium sulfate with radioactive iodide at 120–160 °C (below the melting point of the substrate or of ammonium sulfate) for 1–4 h.[194,195] The authors suggest that this causes a gradual increase in the acidity of the exchange medium via in situ decomposition of the ammonium sulfate with loss of ammonia. This is substantiated by the observation that sulfuric acid can be successfully substituted for ammonium sulfate to promote the exchange.[194,195] A series of iodobenzylguanidines, the three iodobenzoic acids, and other iodo aromatics have been radioiodinated by the ammonium sulfate method.[195] A related finding is that the iodine exchange of a series of 4-(alkylamino)iodoquinolines is greatly facilitated by the use of their phosphate salts.[196] As the pH went down, the radiochemical yields went up.

C. Exchange for Bromine

Bromine has also been used as a leaving group for radioiodination. The advantage of iodine for bromine exchange is that very high specific activities can be achieved, provided the radioiodinated product can be separated efficiently from the brominated precursor. A great deal of work has been done in this area to develop labeling procedures for long-chain fatty acids radioiodinated on the terminal carbon that are used in heart imaging. Others have succeeded in doing aromatic iodine for bromine exchanges. Compounds labeled in this way are shown in Table IX.

One study compared four different methods for making radioiodinated 17-iodoheptadecanoic acid.[201] Iodine for bromine exchange worked both in acetone reflux and in the melt. A drawback to the melt exchange was that at least 100 mg of the bromo acid was needed to obtain a good radiochemical yield. Heptadecanoic acid 17-tosylate was also successfully employed as a substrate for radioiodination. The fourth method used a two-phase system with iodoheptadecanoic acid as the substrate and tributyl hexadecylphosphonium bromide as the phase transfer agent. Since the tosylate was much more readily separated from the iodo fatty acid than from the corresponding bromo compound, it was the method of choice for the preparation of large quantities of no-carrier-added ^{123}I 17-iodoheptadecanoic acid.[201]

In the case of 16α-iodoestradiol, extremely high purity of the 16β-bromo precursor is essential for obtaining very high specific activity.[206] The problem was found to be due to a slight contamination of the 16β-bromoestradiol by the 16α-bromo compound. This points up a difficulty encountered in using iodine for bromine exchange to label receptor-binding compounds. It is

Table VII. Compounds Labeled by Use of Prosthetic Groups and Chloramine T

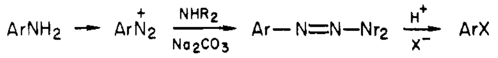
compound	bridge[a]	prothesis[a]	ref
	Steroids		
digitoxigenin	3β-HS	TME	112
	3β-amino	TA	113
estradiol	3- or 17β-urethran	bovine serum albumin	114
	7-CMO	HA	115
	7-CMO	TA	116
	6-CMO	HA	14
	6-CMO	TME	117
	17β-HS	TME	117
estriol	6-CMO	HA	118
testosterone	17β-HS	TME	119
	11α-HS	HA	14
	3-CMO	HA	114, 119
	3-CMO	TME	120, 121
	3-CMO	HA	118, 122
progesterone	3-CMO	HA	118
	11α-HS	TME	123
	11α-HS	HA	14, 122
	3-CMO	HA	114, 122
	11α-hemiphthalate	TME	124
	11α-carbonate	TME	125
	11α-HS	TME	124, 125
	11α-HS	TA	124
	11α-HS	HA	124
	3-CMO	TA	124
	12-CMO	TA	124
	20-CMO	HA	124
cortisol	21-HS	TME	119
	3-hydrazone	TA	126
	3-CMO	histidine	127
aldosterone	3-hydrazone	TA	126
	21-HS	TME	119[b]
cholylglycine	terminal CO_2H	HA	128, 129
	terminal CO_2H	TA	129
chenodeoxycholic acid	terminal CO_2H	HA	129
glycochenodeoxycholic acid	terminal CO_2H	HA	129
norgestrel	3-CMO	TME	130
norethisterone	3-CMO	TME	130
	3-CMO	HA	14, 122
	11-HS	HA	14, 122
β-ecdysone	6-CMO	TA	131
20-hydroxyecdysone	20-HS	TME	151
androstenedione	19-hydroxyl	2-(4-hydroxyphenyl)acetate	132
	19-methoxycarboxyl	TA	132
	Prostaglandins		
E_1	terminal CO_2H	TA	133
	terminal CO_2H	HA	133
	terminal CO_2H	histidine	134
E_2	terminal CO_2H	TA	133
	terminal CO_2H	HA	133
	terminal CO_2H	histidine	134
$F_{1\alpha}$	terminal CO_2H	histidine	134
$F_{2\alpha}$	terminal CO_2H	TA	133
	terminal CO_2H	HA	133
	terminal CO_2H	histidine	134
	terminal CO_2H	TME	135
	Other Compounds		
pindalol	replace H of CH_3	4-hydroxyphenyl	136, 137
2-((ethylamino)methyl)tetralone	replace H of CH_3	4-hydroxyphenyl	138
practolol	replace isopropyl	4-hydroxyphenethyl	139
alprenolol	replace isopropyl	4-hydroxyphenethyl	139
inulin	ether	allyl	140[c]
	ether	propargyl	141[c]
cAMP	2′-HS	TME	142, 143, 144[d]
cotonine	4′-CO_2H	TA	145, 152
limonin	7-CMO	TME	146
tetracycline	2-carboxyl	*N*-(methylamino)pyrazole	147[e]
biotin	2,6-hexanediamine hemiamide	BH	148
methotrexate	terminal CO_2H	TME	149
	terminal CO_2H	HA	153, 154
	ethylenediamine hemiamide	BH	153, 154
melatonin	replace acetyl	BH	150[f]
bleomycin		BH	69
clonazepam		BH	155, 156

TABLE VII (*Continued*)

compound	bridge[a]	prosthesis[a]	ref
diphenylhydantoin	3-*N*-(acetic acid)	TME	157
anileridine	HS	TA	158
normeperidinic acid	terminal CO_2H	TA	158
n-butylbiguanide	replace CH_2CH_3	4-hydroxyphenyl	159
dibenzo-*p*-dioxin		5-iodovaleramide	160[e]

[a] HS = hemisuccinate; CMO = (carboxymethyl)oxime; TME = tyrosine methyl ester; TA = tyramine; HA = histamine; BH = Bolton-Hunter reagent (3-(4-hydroxyphenyl)propanoate). [b] Labeled by electrolysis. [c] Labeled by addition of molecular iodine. [d] Labeled by use of lactoperoxidase. [e] Labeled by iodine exchange. [f] Labeled by use of H_2O_2.

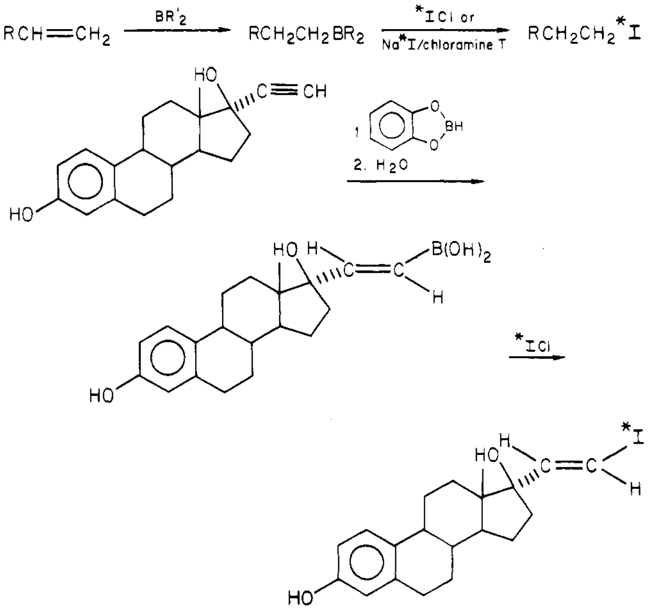
SCHEME IV. The Wallach Reaction



$$ArNH_2 \rightarrow Ar\overset{+}{N}_2 \xrightarrow[Na_2CO_3]{NHR_2} Ar{-}N{=}N{-}NR_2 \xrightarrow[X^-]{H^+} ArX$$

X = F, Cl, Br, I

SCHEME V. Radioiododeboronation



likely that the bromo compound will bind to the particular receptor as well as or better than the iodo compound; any bromo compound present in the final preparation results in a lowering of the effective specific activity.

D. Catalyzed Exchange

Efforts to improve radiochemical yields and decrease reaction times for exchange reactions, especially important for ^{123}I, have led to the examination of catalysts for the isotopic exchange reaction. Polymer-supported phosphonates have been shown to catalyze the exchange between radioactive sodium iodide and alkyl halides.[209] Similarly, the presence of dicyclohexyl-18-crown-6 catalyzes the exchange of labeled sodium iodide with alkyl bromides.[210]

Several reports concerning the use of copper to catalyze radioiodide exchange have appeared. Copper metal or copper(I) salts have been reported to catalyze the exchange of iodo aromatics with radioiodide in dimethyl sulfoxide.[211] Similar results with copper(I) salts have been reported in the exchange of 2-iodohippuran[212] and of iodinated estradiol 3,17-bis(phosphates).[213]

The role of copper salts in the labeling of 2-iodohippuran[212] has been explained in terms of coordinating with the 2-iodobenzoic acid impurity present in commercial 2-iodohippuran and thus preventing its preferential labeling.[214] If 2-iodohippuran of sufficient purity is used (>99.9%), copper salts are not required.[214]

An exchange between radioiodide and hydrogen to form labeled 4-iodoantipyrine has been shown to be catalyzed by silica gel.[215] The mechanism of this reaction is unknown.

E. Iododediazonization

The treatment of aromatic diazonium salts with inorganic iodide to produce the corresponding aromatic iodide is a standard laboratory procedure.[216] The approach is obviously an attractive one for the preparation of aromatic compounds labeled with radioiodine. Two things are necessary. The appropriate aniline must be available, and other functional groups in the molecule must be stable to (or protected from) reaction with nitrous acid or alkyl nitrite used to form the diazonium salt. The Gatterman reaction is a variation of the standard iododediazonization procedure, which employs copper-bronze to catalyze the reaction.[216]

Recently, the Wallach reaction[225] (Scheme IV) has been resurrected for aromatic radiohalogenation. In this reaction the diazotized amine is trapped by formation of a triazene with an appropriate secondary amine. The triazenes are stable, isolable solids that when reacted with inorganic halides under acidic conditions give aromatic halides. Although it was used for radiofluorination at first,[226,227] it has since been demonstrated to be even more useful for iodination[228] and radioiodination.[229] Table X shows compounds that have been radioiodinated via various iododediazonization procedures.

F. Iododeboronation

It has been known for some time that organoboranes react with molecular iodine under basic conditions to produce alkyl or vinyl iodides with retention of configuration.[232] This chemistry has recently been adapted for use in radioiodination. The use of molecular iodine has several disadvantages in radiolabeling, as discussed above. Therefore, reaction conditions have been developed for the iododeboronation reaction that employ iodine monochloride[233] or sodium iodide and chloramine T[234] (Scheme V). This chemistry is useful for labeling alkyl compounds provided the corresponding alkene derivative is available.[235,236] Labeled vinyl iodides can be made from the appropriate alkynes via vinylboronic acid intermediates.[237] Labeled iodobenzene has been made from triphenylborane, but only one of the three

TABLE VIII. Compounds Labeled by Iodine Exchange in Solvent

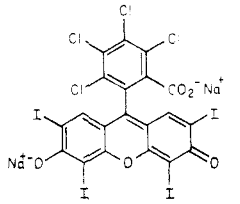
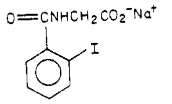
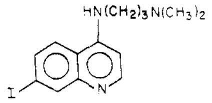
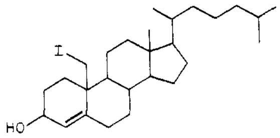
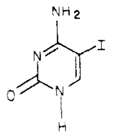
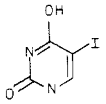
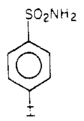
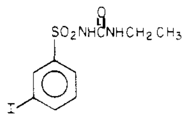
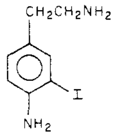
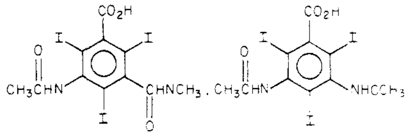
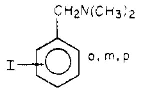
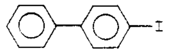
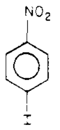
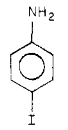
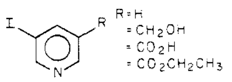
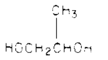
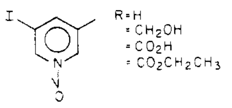
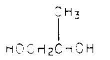
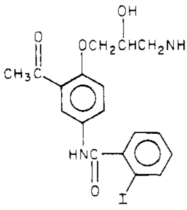
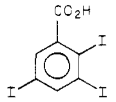
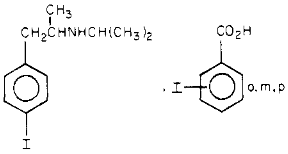
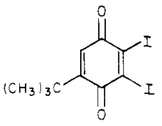
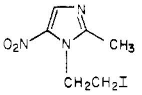
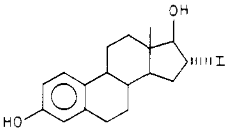
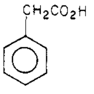
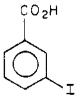
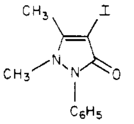
compound	solvent	ref
	H_2O EtOH/HIO_3	168 169
	H_2O or EtOH	170
	$HOCH_2CH_2OH$	171
	2-propanol or acetone	172
 	H_2O	173
	$HOCH_2CH_2OH$	174
	Me_2SO	175
	EtOH	102
	H_2O	176
	aqueous NH_4OH	177
	$HOCH_2CH_2OH$	178
 	$HOCH_2CH_2OH$	178
 R=H =CH₂OH =CO₂H =CO₂CH₂CH₃	 $HOCH_2\overset{CH_3}{\overset{\vert}{C}}HOH$	179
 R=H =CH₂OH =CO₂H =CO₂CH₂CH₃	 $HOCH_2\overset{CH_3}{\overset{\vert}{C}}HOH$	179

TABLE VIII (*Continued*)

compound	solvent	ref
	H_2O	180, 181
	HOAc	182
	HOAc	183

[a] R = H, CH_2OH, COOH, $CO_2CH_2CH_3$.

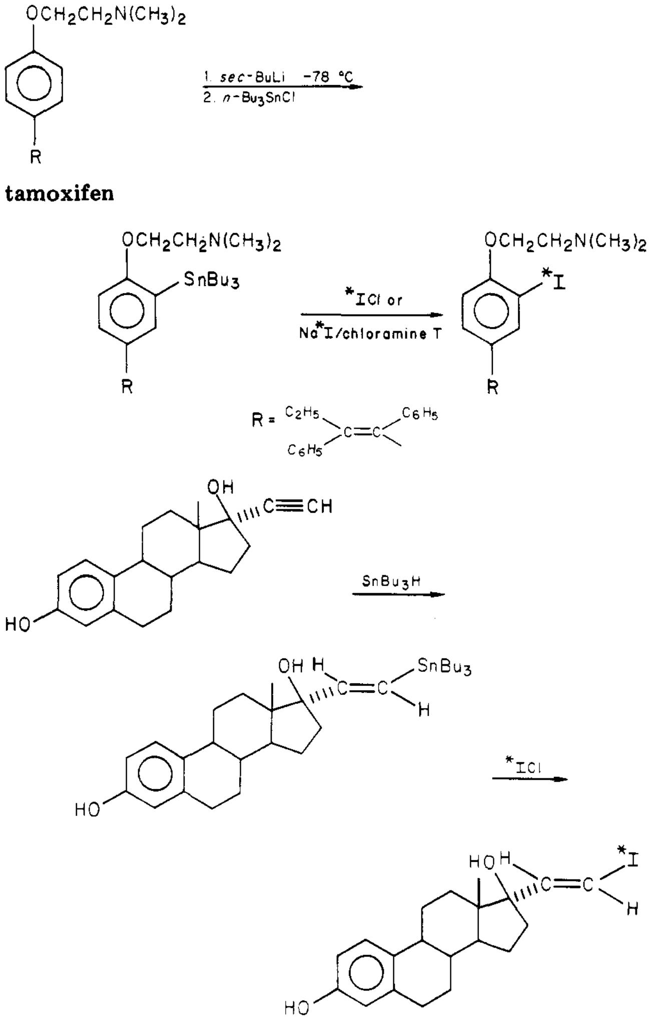
TABLE IX. Compounds Labeled by Iodine for Bromine Exchange

compound	method	ref
$I(CH_2)_6CH{=}CH(CH_2)_7CO_2H$	acetone reflux	53
$I(CH_2)_5CO_2H$	acetone reflux	53
$I(CH_2)_{10}CO_2H$	acetone reflux	53
$I(CH_2)_{15}CO_2H$	acetone reflux	198
$I(CH_2)_{16}CO_2H$	acetone reflux	197 199 200 201
$I(CH_2)_{16}CO_2H$	DMF/80 °C	200
$I(CH_2)_{16}CO_2H$	melt	201
$I(CH_2)_{16}CO_2H$	acetone/100 °C/ sonicate	202
	H_2O reflux	203
	acetone reflux	204
	acetonitrile reflux	205 206
	$(NH_4)_2SO_4$ melt	195
	acetamide melt	190
	aqueous acid	2
$I(CH_2)_8Te(CH_2)_7CO_2H$	acetone reflux	208

phenyl groups is used.[234,237]

Radioiodination via organoborane chemistry has been used to prepare labeled ω-iodo fatty acids[235,238-240] and iodovinylestradiol,[237,239,241] which was made from ethynylestradiol (Scheme V).

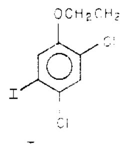
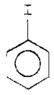
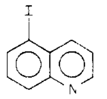
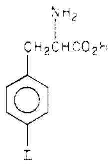
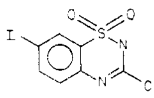
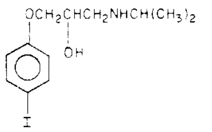
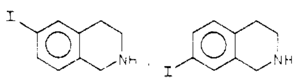
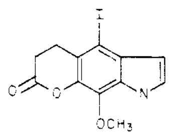
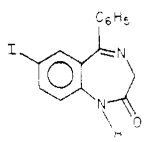
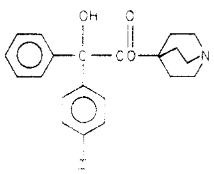
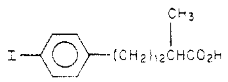
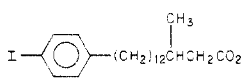
SCHEME VI. Radioiododestannylation



G. Iododestannylation

The tin–carbon bond in organotin compounds can be cleaved by halogens. This cleavage is selective for aromatic groups over alkyl groups.[242] This fact has been used to develop a radioiodination technique for aromatic and vinyl substrates. The required tri-*n*-butyltin compounds can be made from lithiated organics or, in

TABLE X. Compounds Labeled By Iododediazonization

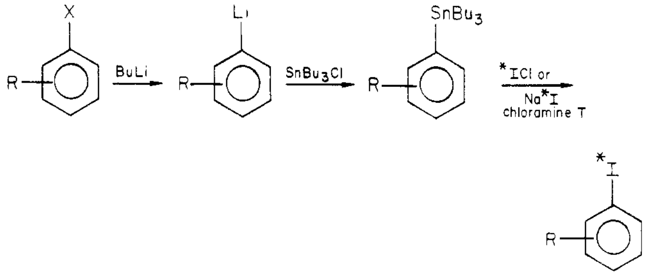
compound	reaction[a]	ref
	S	217
	S	218
	S	219
	S	220
	S	221
	G	222, 223
	G	224
	W	229
	W	229
	W	230
	W	231
	W	231

[a] S = standard, G = Gatterman, W = Wallach.

the case of vinyl compounds, by reduction of the corresponding alkyne with tri-*n*-butyltin hydride (Scheme VI). These compounds are then treated with labeled iodine monochloride or sodium iodide and chloramine T to produce the radioiodinated derivatives. Compounds labeled by iododestannylation are tamoxifen,[243-245] an antiestrogen (see Scheme VI), and iodovinylestradiol.[246]

In the case of tamoxifen the (dimethylamino)ethoxy side chain was used to direct aromatic lithiation.[243] Since aromatic lithiation can also be done by halogen–metal interchange,[247] it is possible to start with an aromatic bromide or iodide and obtain the corresponding radioiodinated compound (Scheme VII). Thus, what is in a formal sense an exchange is capable of producing high specific activity.

SCHEME VII. A High Specific Activity "Exchange" Reaction via Radioiododestannylation



X = Br, I

H. Iododesilation

The carbon–silicon bond bears some similarity to the carbon–tin bond. Thus it is not surprising that molecular halogens cleave the carbon–silicon bond readily[248] or that organosilanes are being used for radioiodination. Aromatic trimethylsilanes on treatment with labeled sodium iodide and *tert*-butyl hypochlorite in acetic acid at 60 °C give good yields of the corresponding radioiodinated product.[249]

I. Iododethallation

Thallium(III) trifluoroacetate electrophilically thallates aromatics, and the resulting arylthallium bis(trifluoroacetate) can be treated with inorganic iodide to produce aromatic iodides.[250,251] The substitution is predominantly para except in the case of strong electron-withdrawing groups where it is meta and benzoic acid where it is ortho.[250,251] This organothallium chemistry is beginning to find use in radioiodination. An aromatic derivative of cellulose[252] and two phenyl-fatty acid analogues[253,254] have been radioiodinated this way.

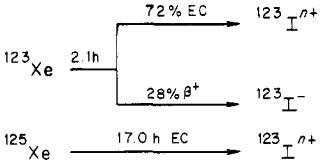
A procedure that may involve similar chemistry has been developed for the radioiodination of cytosine and deoxycytosine residues in DNA or RNA[9,255] or by themselves.[256,257] The nucleoside (or single-stranded nucleic acid) is treated with thallium(III) chloride in the presence of radioactive iodide. The reaction produces labeled 5-iodocytosine.

IV. Other Methods of Radioiodination

A. Sodium Iodide Melt

A very simple, but intriguing, method of radioiodination involves simply heating labeled sodium iodide with a melt of the noniodinated compound to be labeled. It was originally developed for estradiol.[258] It is not an exchange, because estradiol is not an iodinated compound. Chromatography indicated that a mixture of 2-iodo- and 2,4-diiodoestradiol had been formed.[258,259] The mechanism of this reaction is unknown. Both testosterone and dihydrotestosterone gave mixtures of unknown labeled materials by this method.[259,260] In the case of testosterone, only one of the three labeled products produced was immunoreactive with antibodies

SCHEME VIII. Production of Positive Iodine Species by Decay of Xe Parents[a]



[a] EC = electron capture

raised against testosterone; its structure was not determined.[260] The reaction must involve some sort of oxidation/reduction, but its mechanism awaits further study.

B. Recoil Labeling

Both ^{123}I and ^{125}I can be obtained from the decay of the corresponding Xe isotopes (Scheme VIII). The production of highly positive iodine species (recoil atoms) as a result of the electron capture decay and ensuing loss of Auger electrons has been used for radiolabeling.

When serum albumin was exposed to ^{123}Xe for 6–12 h at liquid nitrogen temperature, 80% of the iodine produced was protein bound.[261] Insulin treated with radioxenon and KIO_3 as a catalyst gave 50% and 80% yields, respectively, of [^{123}I]- and [^{125}I]monoiodoinsulin.[262] Deoxyuridine and L-tyrosine were labeled in the same way in the very good yields.[262] An iodinated quinoline derivative labeled with ^{123}I has been prepared in modest yield by recoil labeling.[263] The reactions of radioactive recoil atoms with aromatic compounds have been reviewed recently.[264] This method does require access to a cyclotron and associated facilities.

A related procedure produces ^{128}I-labeled compounds by neutron activation of the corresponding stable iodinated compounds in an ice lattice.[265] The ^{128}I atoms produced by the neutron bombardment recombine with the aromatic ring because they are held in close proximity by the ice. This method cannot produce material of high specific activity or labeled with any of the more useful iodine isotopes.

V. Discussion

A. Determining the Structure of Radioiodinated Compounds

There are three cases in which it is possible to predict with a good degree of certainty the structure of the product of a radioiodination reaction. If the starting compound contains an activated aromatic ring such as a phenol, aniline, or imidazole and is treated with a source of electrophilic iodine, then speculation as to the location of the radioiodine in the final product can be made with some justification on the basis of model compounds such as tyrosine and histidine. If the compound is labeled by an exchange reaction with sodium iodide, then the label should appear in the place of the leaving group. If the labeling reaction uses some other leaving group such as iodine for bromine exchange, iododediazonization, or iododemetalation, then the radioiodine should again appear in the place of the leaving group.

Nevertheless, all of those structural assignments are made on the basis of analogy with nonradioactive reactions. The only physical property of a no-carrier-added radioiodinated material that can readily be directly measured is its behavior in various forms of chromatography. The number of molecules present in a sample of a radioiodinated compound of high specific activity is just too small and the half-life often too short to make use of such things as NMR and IR spectra for structure elucidation. Therefore, it is a big advantage to have a known sample of the ^{127}I compound on hand so that its chromatographic behavior can be compared to the compound labeled with ^{123}I, ^{125}I, or ^{131}I. The stable compound can be prepared by scaling up the radioiodination reaction or by an alternate route. The former has the advantage of corroborating the regiospecificity of the radioiodination reaction.

Now it is possible to treat something with radioactive sodium iodide and some form of oxidizing conditions (i.e., a pinch of chloramine T[266]) and obtain a labeled product. It may serve its purpose, binding to an antibody in a radioimmunoassay or localizing in tumor as a radiopharmaceutical. Still, if no one knows what it is, the labeled material is good only for that purpose and adds nothing to the store of structure–activity knowledge. And it is that kind of knowledge which is needed to deal with problems like bridge binding in RIA and the design of new agents for nuclear medicine.

B. Summary

Radioiodinated compounds are useful in biology and medicine. The chemistry of radioiodination has been developed to a point where there are a large number of labeling methods to choose from.

The earliest methods, derived from protein labeling work, are oxidative in nature. They make use of an oxidizing agent and labeled sodium iodide to produce some electrophilic iodine species that is capable of reacting with an activated aromatic moiety present in the compound to be labeled. If such a group is not present, it can be attached as a prosthetic group. These methods are capable of producing labeled material of high specific activity, but side reactions due to the oxidizing agent are always a potential problem. These side reactions have been minimized by using milder oxidizing conditions and solid-phase oxidizing agents or by labeling a prosthetic group and attaching it to the target compound in a separate reaction.

An alternative approach to radioiodination is the replacement reaction. In this case an existing part of the molecule is activated toward radioiodination by the attachment of an appropriate leaving group. The choices include iodine itself, bromine, a diazotized aniline, and derivatives of boron, tin, silicon, and thallium. At its simplest, the iodine for iodine exchange, it is not possible to prepare high specific activity material by this method. However, when a leaving group other than iodine is used, the limitation becomes the ability to separate the radioiodinated product from unlabeled starting material. High-performance liquid chromatography has become the tool of choice for this purpose. The choice of the leaving group to employ will be made on the basis of the specific activity required in the product and the chemical nature of the compound to be labeled.

Many of the older oxidative methods are still very useful. The scope and the limitations of replacement type reactions is under exploration. It should be possible to find a technique suitable for the radioiodination of most any small organic molecule.

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