Translated from *Uspekhi Khimii*, 50, 196-223 (1981) U.D.C. 541.123.52:541.128:546.11.03:547.9

The Introduction of Tritium into Biologically Active Compounds with the Aid of Exchange Methods

L.A.Neiman

Different versions of the homogeneous and heterogeneous catalytic isotope exchange as well as catalytic substitution of a halogen by tritium are examined from the standpoint of their use for the introduction of a tritium label into compounds of biological interest (aminoacids, peptides, proteins, nucleotides, carbohydrates, steroids, medicinal agents, etc.). The bibliography includes 214 references.

CONTENTS

I.	Introduction	109
II.	Exchange of labile hydrogen atoms	109
III.	Homogeneous catalysis of hydrogen isotope exchange	110
IV.	Heterogeneous catalysis of hydrogen isotope exchange	113
V.	Catalytic dehalogenation	117

I. INTRODUCTION

Biology and related disciplines—biochemistry, molecular biology, and bio-organic chemistry—are fields of study where the use of isotope exchange has proved most fruitful. The present development of a whole series of branches of these sciences could not be imagined without the utilisation of radioactive isotopes and some branches (for example, the study of metabolism or biogenesis) have been placed on a genuine scientific basis solely as a result of the application of the isotope method. Isotope exchange has now come to be widely used in biochemistry and bio-organic chemistry as a method for the qualitative and quantitative analysis of substances. The reason for this is that isotope exchange is more sensitive by 4–6 orders of magnitude than spectroscopic methods and moreover in most instances does not require careful purification of the substances to be determined. It is frequently actually unnecessary to isolate them or to comminute the sample in which they are contained. The high sensitivity of the isotope method is particularly important for biological disciplines, because it permits a reliable determination of the substances at concentrations close to those at which their biological activity is shown.

Since the sensitivity of the method is unambiguously related to the specific radioactivity a of the labelled compound, there has arisen in recent years a clear tendency to use tritium-containing organic compounds instead of their analogues labelled with ^{14}C. Indeed, when a single T atom is introduced into a molecule, a ~30 c mmol^{-1} should be attained theoretically, while the introduction of a single ^{14}C atom yields only 64 μc mmol^{-1}. Since the molecules of organic compounds contain in many instances tens of hydrogen atoms, it is in principle possible to obtain tritiated compounds with a amounting to several hundreds and more of c mmol^{-1}. However, in practice preparations with $a > 50$–100 c mmol^{-1} are rarely used, because under the conditions of high values of a autoradiolysis is accelerated. Other advantages of tritium are the possibility of introducing it by exchange methods (i.e. into a "finished" molecule) and a much lower cost of tritium-containing compounds compared with those labelled with ^{14}C. As regards other radioactive isotopes employed (^{32}P, ^{33}P, ^{35}S, ^{125}I, etc.), the region of their applicability is naturally much narrower.

Thus tritium is nowadays the most important and most common radioactive isotope used in biology and related disciplines and the problem of methods of synthesising tritium-labelled biologically active substances (BAS)† has become extremely urgent. These methods include chemical synthesis, biosynthesis, synthesis with the aid of hot atoms, and exchange methods (catalytic isotope exchange, the Wilzbach method and its modifications, etc.), the exchange methods having a number of advantages particularly in the preparation of labelled BAS of complex structure—these are primarily single-stage synthesis ("rapidity" of synthesis), universality, comparative simplicity, and the possibility of obtaining high values of a.

This review is devoted to exchange methods for the preparation of tritium-containing BAS; the exchange of labile hydrogen atoms and homogeneous and heterogeneous catalysis of the hydrogen–tritium exchange are considered in detail. Furthermore, the review includes the method of catalytic dehalogenation, which is strictly speaking one of the synthetic methods. This is because of, firstly, its similarity (both from the standpoint of the mechanism and from the standpoint of the purely preparative aspect) to the heterogeneous catalysis of isotope exchange and, secondly, because of the extremely great importance which the method has assumed (particularly in recent years) for the preparation of virtually all classes of labelled BAS, in the first place peptides, proteins, and nucleotides. The Wilzbach method and its modifications are not considered in this review. Attention has been concentrated on studies published in the last ten years; a compilation of earlier literature can be found in Evans's monograph published in Russian translation[1] in 1970. Studies on deuterium are quoted only in those cases where it is of interest to compare them with the corresponding results for tritium or when such results for tritium are not available at all but the latter could be obtained (judging from the studies using deuterium).

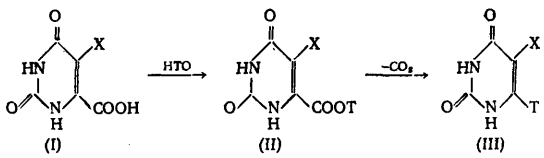
II. EXCHANGE OF LABILE HYDROGEN ATOMS

On dissolution, compounds containing the >NH, –OH, and –SH groups in protic tritium-containing solvents (HTO, MeOT, EtOT, AcOT, etc.) are known to exchange rapidly the H

† In the present review biologically active substances (BAS) are understood to be both products of natural origin (proteins, peptides, aminoacids, carbohydrates, nucleotides, steroids, etc.) and synthetic preparations (medicinal agents, pesticides, carcinogens, etc.).

atoms of these groups (labile hydrogen atoms) for T even under the usual conditions. If the solute is capable of undergoing tautomeric transformations (for example keto–enol or imine–enamine tautomerism), the H atoms linked to the C atoms can also exchange with the tritium-containing solvent under conditions (frequently fairly mild) under which a tautomeric equilibrium is established (i.e. the stable H atoms become labile).

The introduction of tritium in a labile position takes place readily and reversibly, so that the reaction is as a rule of no preparative value, since the labelled BAS obtained by this method lose tritium completely on dissolution in the usual solvents or on being employed in biological studies. However, in certain cases tritium can be fairly simply transferred from a labile to a stable position. An excellent example is provided by the synthesis of 5-fluorouracil-6-*t* [2–5] and 5-bromouracil-6-*t* [6] from the corresponding orotic acids (I), which readily exchange their carboxyl hydrogen on heating with HTO or (the preferred method) [4,5] with a solution of T_2O in dioxan or dimethylformamide (DMF) (100 °C, 1 h). Such solutions are obtained as a result of passing T_2 through a suspension of PtO_2 in dioxan or DMF. Labelled orotic acids (II) are then decarboxylated at 250–300 °C with formation of the corresponding tritiated nucleic bases (III) having $\alpha \approx 1$–2 c mmol^{-1}. It has been shown[7] that thymine-6-*t* can be obtained similarly:



(I) (II) (III)

X = Br, F, or Me.

The same principle constitutes the basis of a method for the specific introduction of D or T into the α-position in α-aminoacids (alanine, phenylalanine, glutamic acid, and aspartic acid)[8]. The method involves the isotope exchange of the carboxyl hydrogen in the corresponding *N*-acylaminomalonic acid (IV) with D_2O or HTO and subsequent hydrolysis and decarboxylation. All three stages can be carried out in a single experiment:

$$\underset{(IV)}{RCONHCR'(COOH)_2} \xrightarrow{HTO} RCONHCR'(COOT)_2 \xrightarrow[HTO]{\Delta} RCOOH + H_2NCT(R')COOH.$$

III. HOMOGENEOUS CATALYSIS OF HYDROGEN ISOTOPE EXCHANGE

Both base and acid catalysis as well as catalysis in the presence of soluble transition metal salts have been used to introduce tritium into BAS.

1. Base Catalysis

In base catalysis the isotope exchange process is initiated as a result of attack by a B^- anion, which abstracts a proton from the compound involved in exchange with formation of a carbanion, which then combines with T^+. If the intermediate carbanion is an ambident species [for example, (V)], i.e. can be protonated at two (or more) centres, the conditions for isotope exchange are the same as the conditions for tautomeric transformations and the latter can be frequently identified from the occurrence of isotope exchange:

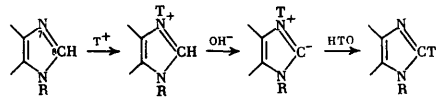
$$R_2CH-C(R')=X \xrightarrow{B^-} [R_2C\cdots C(R')\cdots X]^- \xrightarrow{T^+} R_2CT-C(R')=X+$$
(V)

$$+ R_2C=C(R')-XT \quad ;$$

X=O, N–, C .

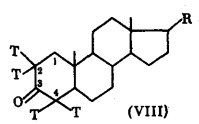
In certain cases the deprotonation proceeds very readily. Thus purine bases, nucleosides, and nucleotides exchange hydrogen, albeit slowly, at $C_{(8)}$ even at pH 8 and room temperature[9,10]. In the preparative synthesis of labelled purines it is necessary to heat the latter with HTO at 85–100 °C; the reaction time varies from 20 min (for DNA and nucleoside triphosphates, which undergo hydrolysis under these conditions) to several hours[11–13]. The rate of exchange is determined by both electronic and steric factors. The presence of a ribofuranosyl residue facilitates the exchange: at 85 °C adenosine exchanges at $C_{(8)}$ twice as fast as adenine which, however, exchanges hydrogen at $C_{(2)}$ 2000 times faster than at $C_{(8)}$.[14]

The conformation of the macromolecule and the microstructure of the helix which determine the accessibility of a particular section of the nucleic acid molecule to water, exert a significant influence[15–18]: the exchange of H at $C_{(8)}$ with HTO in the purine residues of a natural double helix DNA is almost three times slower than for a mixture of the corresponding polynucleotides; the double helix RNA exchanges even more slowly (by a factor of 30–40). Studies of the kinetics of the exchange at different pH have shown[19–22] that in reality the $N_{(7)}$-protonated form (VI) and not the purine molecule itself is deprotonated. The former is formed very rapidly, while its conversion into the ylid (VII) is the rate-limiting stage in the entire process:



(VI) (VII)

Methylene groups activated by neighbouring electron-accepting groups (>CO, –COOR, –CN, etc.) exchange with HTO at alkaline pH, usually with heating. For ketones, for example camphor[23], the exchange proceeds under conditions identical with those for enolisation, and the isotope exchange mechanism is in this case identical with the mechanism of tautomeric keto–enol transformations. In order to introduce T into the α-position relative to the oxo-group in testosterone, progesterone, and other 3-oxosteroids, a convenient preparative method has been proposed[24,25]. It consists in passing a solution of the test substance in an organic solvent through a column filled with basic alumina subjected to a preliminary treatment with HTO. Tritium has been introduced into the 2- and 4-positions of 3-oxo-5α-cholestan-26-yl acetate (VIII) by a similar procedure—isotope exchange with iso-PrOT in the presence of aqueous NaOH:[26]



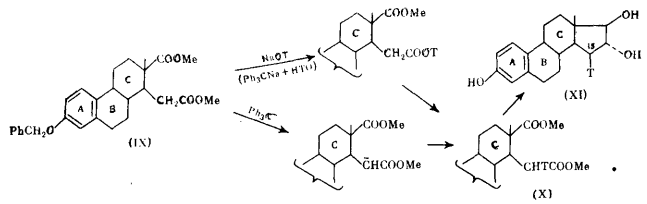
(VIII)

The tritium atoms introduced by these procedures into the steroid molecule are entirely stable in acid and neutral media, but at pH > 10 they exchange for H to the extent of 80–95% even at the usual temperature over a period of 24 h.

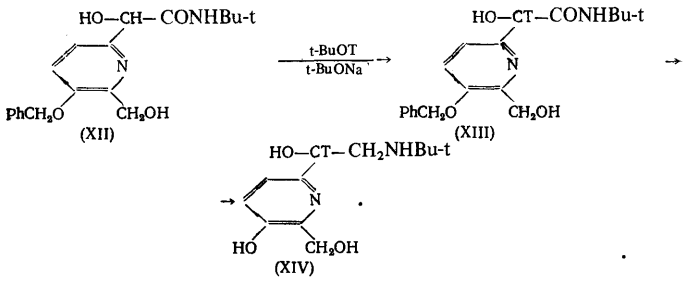
Isotope exchange involving the hydrogen in a methylene group activated by neighbouring carbonyl groups proceeds readily in an alkaline medium also in aminosugars (pH 11–11.5; 20 °C; 48 h), as shown for example, for 2-acetamido-2-deoxy-D-glucose[27], where deuterium can be introduced in the 2-position by this procedure with a high yield. Since alkaline epimerisation also takes place under these conditions 2-acetamido-2-deoxy-D-mannose-2-C-*d* is formed as a side product.

Steroid lactones—cardiac glycosides of the digitoxin series and their aglycones—exchange with HTO in DMF in the presence of Et_3N (30–80 °C, 2–8 h), more than 95% of tritium being incorporated in the lactone ring. Tritium-labelled digitoxin, digitoxigenin, digoxin, gitoxigenin, and uabain with $a \cong 0.4$–0.7 c mmol^{-1} have been obtained by this method[28].

Homogeneous base catalysis has also been used in the synthesis of triol-15-*t* (XI).[29] The 3-benzilate of the dimethyl ester of marianoleic acid (IX) is tritiated in the α-position relative to the methoxycarbonyl group on successive treatment with triphenylmethylsodium and HTO and the resulting labelled ester (X) was converted by acyloin condensation and subsequent reduction into 15-tritioestriol (XI); this compound contains a tritium atom in a position within the molecule from which it cannot be removed by the water present in living organisms:



The isotope exchange between the *N*-t-butylamide of 2-(5-benzyloxy-6-hydroxymethyl-2-pyridyl)-2-hydroxyacetic acid (XII) and t-BuOT in the presence of t-BuONa takes place readily (20 °C; 16 h). The resulting amide-α-*t* (XIII) serves as the starting material for the synthesis (reduction with B_2H_6 and subsequent catalytic debenzylation) of the labelled medicinal preparation pirbuterol (XIV):[30]

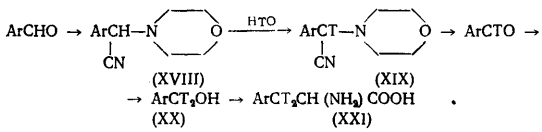


The hydrogen atoms of the α-methylene group are mobile not only in lactones, esters, and acid amides (as can be seen from the examples quoted above) but also in the carboxylic acids themselves. The sodium or potassium salts of aliphatic acids (ranging from butyric to stearic) are converted in high yields into salts of α-labelled acids (XVI) and (XVII) on heating with NaOH and D_2O or HTO under severe conditions (autoclave; 200 °C; 1–3 days) with intermediate formation of the corresponding carbanions (XV):[31]

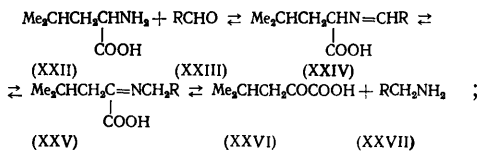
$$RCH_2COOK \rightarrow \overline{R}CHCOOK \rightarrow RCHTCOOK \rightarrow RCT_2COOK$$
$$\text{(XV)} \qquad \text{(XVI)} \qquad \text{(XVII)}$$

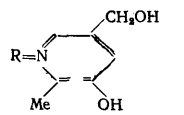
The α-hydrogen atoms in lactic[32], chrysanthemic[33], and abscisic[34] acids exchange for *D* or *T* under similar conditions and in the last case tritium also enters the 4-position and the methyl group linked to $C_{(5)}$; the preparation obtained has a high activity $a \cong 6.5$ c mmol^{-1}.

The isotope exchange involving aminoacids is unsatisfactory under these conditions. Labelled aminoacids are therefore prepared by other, more complex methods; some of these also involve isotope exchange catalysed by bases. For example, under the influence of morpholine and an aqueous NaCN solution, aromatic aldehydes are readily converted into morpholinoacetonitriles (XVIII), which exchange hydrogen in the α-position relative to the cyano-group on heating with D_2O or HTO (DMF or sulpholan; 100 °C; ~5 h).[35] Hydrolysis of the labelled nitriles (XIX) leads to aldehydes containing tritium in the formyl group; on disproportionation of these aldehydes via the Cannizzaro reaction, the corresponding benzyl-α,α-t_2 alcohols (XX) are formed and are then converted by familiar synthetic methods into aromatic aminoacids (phenylalanine, tyrosine, and dihydroxyphenylalanine) labelled with tritium in the β-position [compound (XXI)]:



A novel possibility for hydrogen isotope exchange in aminoacids under the conditions of base catalysis was demonstrated with deuterium for leucine (XXII),[36] which is converted into leucine-α,β,β-d_3 on boiling in D_2O in the presence of pyridoxal (XXIII) and potassium alum. Under these conditions, an equilibrium is established between leucine and pyridoxal, on the one hand, and the ketoacid (XXVI) and pyridoxamine (XXVII), on the other, with intermediate formation of the corresponding Schiff bases (XXIV) and (XXV). The tautomerism of these bases (XXIV) ⇌ (XXV) in D_2O is in fact responsible for the hydrogen exchange in the α-position of leucine, while the exchange in the β-position is in all probability a consequence of the keto–enol tautomerism of the ketoacid (XXVI):





Numerous studies of the kinetics of hydrogen exchange in peptides and proteins (see, for example, Refs. 37–41) as well as polyamides, treated as models of protein[41–44], indicate, in the way already mentioned above for polynucleotides, a considerable influence of the polymer matrix (particularly a protein matrix) on the nature of the transition state and the exchange mechanism. The rate of exchange is determined by the steric accessibility of various sections of the protein molecule to the solvent and is a minimum for the most ordered structures. It has been shown for collagen[45] that, as a result of the rupture of intramolecular hydrogen bonds, the exchange with D_2O is accelerated.

The hydrogen atoms in aromatic compounds are also capable of isotope exchange in an alkaline medium (NaOT or KOT solutions in HTO or in HTO–DMF, MeONa in MeOT, and KNT_2 in liquid NT_3).[46] The exchange is promoted by the presence of methoxy-groups and particularly phenolic hydroxy-groups as substituents in the aromatic ring, which has been used to introduce tritium into the *ortho*- and *para*-positions relative to the hydroxy-group in phenolic aminoacids[47] and in phenolic alkaloids related to morphine[48–51]. Heteroaromatic compounds and their hydro-derivatives also exchange under analogous conditions; the exchange is most effective in liquid NT_3,[46,51] but experimental difficulties in studies using this solvent limit its practical application. The exchange involving nitrogen-containing heterocycles is greatly accelerated when powerful electron-accepting substituents (oxide oxygen or a nitroso-group) are present at the nitrogen atom[46,52,53]. The mechanism of the exchange involves two stages—the formation of a carbanion and its subequent interaction with the labelled medium; both the first stage (in the exchange of methyl hydrogen atoms in ethyl-substituted heterocycles)[53] and the second stage (in the exchange of hydrogen at $C_{(3)}$ in 2-substituted indoles)[54,55] can be rate-limiting.

2. Acid Catalysis

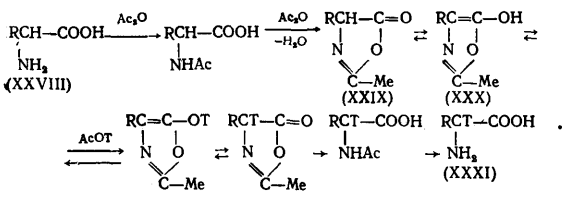
In the homogeneous acid catalysis of hydrogen isotope exchange the intermediate carbonium ion plays the same role as the carbanion in base catalysis (see above). The carbonium ion can be formed either as a result of the abstraction of a hydride ion from the molecule or (more often) as a result of the addition of T^+ or D^+ to an unsaturated carbon atom. The latter version obtains in numerous hydrogen exchange reactions in aromatic systems. The acid agents employed (see the review of Burrish et al.[56]) are strong protic acids (sulphuric, phosphoric, perchloric, and trifluoroacetic), weak acids (for example acetic), Lewis acids ($AlCl_3$, $AlBr_3$, and $EtAlCl_2$), and the complex $H_2TPO_4.BF_3$ (the so-called Yavorsky reagent[57]).

Virtually all these agents have been used successfully to introduce the tritium label into aromatic and heteroaromatic compounds, although in certain cases, involving $HTSO_4$ at elevated temperatures, undesirable side reactions do occur. Howver, tritium has been introduced into *l*-phenylalanine and its *p*-nitrophenyl ester with high yields on heating (90° to 100 °C, 48–72 h) with dilute $HTSO_4$ (or formed from SO_3 and HTO), approximately 95% of the incorporated tritium being contained in the benzene ring and approximately 5% in the β-position[58]. The exchange is accompanied by a slight (<4%) racemisation; the value of *a* for the preparations obtained amounts to several μc $mmol^{-1}$.

In order to obtain other labelled tritiated aromatic aminoacids with *a* of the same order of magnitude, 3–4 *N* solutions of TCl in HTO are most frequently used (heating at 40–90 °C). Under these conditions, 3-hydroxy-*l*-kynurenine exchanges only in the benzene ring[59] and *l*-5-hydroxytryptophan exchanges in the 2-, 4-, and 6-positions in the indole ring[60,61], while the side chains are unaffected and the exchange does not involve racemisation. On raising the temperature (100 °C, several hours), it is possible to achieve the exchange of all the hydrogen atoms in the benzene ring of L-5-hydroxytryptophan[60] and of the hydrogen atom in the 3- and 5-positions of tyrosine and in the 2-, 5-, and 6-positions of 3,4-dihydroxyphenylalanine[47], while it has been shown with the aid of deuterium that in tryptophan[62] the hydrogen atoms in the 2- and 6-positions exchange rapidly and those in the 4-, 5-, and 7-positions exchange slowly only in trifluoroacetic acid. Like tyrosine, 4-aminobenzoic acid also exchanges in the 3- and 5-positions on heating with 3 *N* TCl in HTO (90 °C, 36 h). This preparation with $a = 2.5$ μc mg^{-1} has been used[63] to synthesise the tritium-labelled 4-methylpiperazide of 4-[*N*-(7-trifluoromethylquinolinyl)amino]benzoic acid, which exerts an effect on the central nervous system.

It has also been shown with the aid of deuterium that an acid medium (DCl in D_2O or AcOD) is suitable for the introduction of the label not only into the benzene ring but also into non-benzenoid aromatic and heteroaromatic natural products—azulenes[64,65], indoles (in the 3-position)[66], chlorophylls[67], and porphyrins[68]. In the same medium [DCl (TCl) in AcOD (AcOT) at 120 °C] the α-hydrogen atoms of carboxylic and aminoacids exchange[69] and also certain aliphatic acids, for example stearic[70], exchange with HTO (130 °C, 48 h, *a* reaching 1 c g^{-1} after washing out the labile tritium) without any extra acid additives.

As regards tritium-containing aminoacids (XXXI), the latter are obtained most effectively by refluxing the Ac_2O in AcOT (the reagent is prepared by adding an excess of Ac_2O to HTO) and subsequent treatment with HTO.[71–74] In this case the hydrogen atom in the α-position exchanges exclusively (or almost exclusively) and the exchange is accompanied by racemisation and does in fact occur for the aminoacid (XXVIII) itself but involves the intermediate azlactone (XXIX), which is converted into the tautomeric form (XXX):



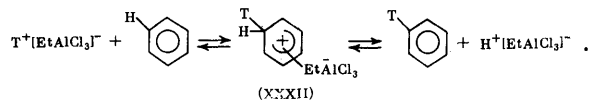
Despite the fact that DL-aminoacids are obtained by this method, in combination with enzymatic resolution of racemates it can be used also to obtain labelled L-aminoacids[73]. An analogous procedure (boiling with Ac_2O into AcOD followed by treatment with D_2O) has recently been proposed as a method for the introduction of deuterium into the α-position in aminoacids[75].

The use of the Yavorsky reagent (the complex $H_2TPO_4.BF_3$) as the tritiating agent for aromatic compounds and compounds containing tertiary hydrogen has a number of advantages (the exchange takes place even at room temperature and there are no side reactions, which leads to radiochemical purity of the products[57,76]); however, owing to the low values of *a* (at most tens of μc $mmol^{-1}$) attainable with its aid, it is hardly used for the preparation of labelled BAS. Insecticides (DDT, parathion, sevin, and their analogues)[77], tropine, and mescalin[78] are exceptions. Recently this list was extended by including insulin ($a \approx 6.5$ μc $mmol^{-1}$ [79] and also certain phenols ($a = 10–80$ μc $mmol^{-1}$), converted as a result of esterification into labelled hypolipidemic agents (including clofibrate)[80].

A modification of the Yavorsky reagent—the complex $AcOT.BF_3$ (prepared by the successive addition of a slight excess of Ac_2O and an equivalent amount of BF_3 to HTO)—was proposed from the very beginning[81] specifically for the introduction of the tritium label into BAS—aminoacids, peptides, proteins (α-lipoprotein, ribonucleoprotein, somatotropin, and insulin), and cholesterol. The exchange takes place under mild conditions (20–24 °C, approximately 20 h), but certain proteins (human albumin and ovalbumin) are

nevertheless denatured and the values of a attained do not exceed 150 μc g^{-1}.

Lewis acids ($AlCl_3$, $AlBr_3$, $MeAlCl_2$, $EtAlCl_2$, WCl_6, $SbCl_5$, and BBr_3) catalyse hydrogen isotope exchange mainly in aromatic compounds and their use for labelled BAS is therefore extremely limited. Best results have been achieved for polynuclear aromatic hydrocarbons—methylcholanthrene, 3,4-benzopyrene, and 9,10-dimethyl-1,2-benzanthracene, which are powerful carcinogens and are widely used in oncological research. After being kept with HTO and $AlCl_3$ in CS_2 for many days (14–50 days at 20 °C), these hydrocarbons are converted into the corresponding generally labelled compounds with $a \cong 20$–50 c mmol^{-1}.[82] The attempts to use $AlCl_3$ as the catalyst of the exchange between HTO and uracil, adenine, and glycine or its peptides (in CH_2Cl_2 at 80 °C) were less successful[83]. Among all the catalysts of this type, the most effective is apparently $EtAlCl_2$, in the presence of which full equilibrium between aromatic hydrocarbons is attained for benzene and its homologues and derivatives after only 30 min at 20 °C.[84] In this case the TCl formed as a rsult of the partial hydrolysis of the catalyst plays the role of a cocatalyst: the strong acid $T^+[EtAlCl_3]$, obtained from TCl and $EtAlCl_2$, adds to the aromatic ring to form the complex (XXXII). The equilibrium between the hydrocarbon, the acid, and the complex is responsible for the hydrogen exchange[85]:



(XXXII)

The catalysis by other Lewis acids proceeds via a similar mechanism. It has been suggested[86] that the HTO–$EtAlCl_2$ system be used for the introduction of tritium into alkanes and cycloalkanes (including steroids) containing at least one tertiary hydrogen atom. However, in order to attain satisfactory values of a, a fairly high temperature (96 °C) is necessary, which also promotes skeletal isomerisation reactions in the intermediate carbonium ion, which leads to the formation of labelled side products. For steroids or other BAS, this method has not found any serious applications.

In catalysis by Lewis acids of the reaction involving the exchange of H for D the experimental procedure is simplified, since deuterated aromatic hydrocarbons (usually C_6D_6) can be employed as the labelled medium[84,87].

The hydrogen isotope exchange in an acidic (sometimes weakly acidic) medium, initiated by various types of radiation (ultraviolet radiation[88–92], γ-radiation[93,94], and the intrinsic radiation of HTO with a high value of a [95,96]), constitutes a special variety of homogeneous acid catalysis. Such exchange involves both aromatic[88–92] and aliphatic[93–96] compounds (particularly carbohydrates[94,95]); the initiation is facilitated by the addition to the medium of substances readily forming radicals—potassium iodide[89,90] and mercaptans[94]. In all cases radicals are formed as an intermediate stage: for aromatic compounds as a result of the addition of T$^\cdot$ and for aliphatic compounds as a result of the abstraction of H$^\cdot$. For example, in the exchange between benzene and HTO in an acid medium in the presence of KI irradiation induces primarily the decomposition of the I^- ion into I$^\cdot$ and a solvated electron[89]. The interaction of the latter with the cation H_2TO^+ leads to H_2O and T$^\cdot$, the addition of which to the benzene molecule with subsequent elimination of H from the radical [PhHT]$^\cdot$ is responsible for the incorporation of tritium into the aromatic ring. In a weakly acid medium the solvated electrons can be captured by benzene molecules with formation of the anion $[PhH]^-$, which is converted into the radical [PhHT]$^\cdot$ as a result of the interaction with the tritium-containing medium[90].

On the basis of published data, isotope exchange initiated by irradiation appears to be relatively unsuitable for the preparative synthesis of labelled BAS, mainly because of side reactions. Even for simple compounds, an inversion of configuration is observed as a consequence of the homolysis of the C–H bond of an asymmetric carbon atom. Thus the isotope exchange between (+)-tartaric acid and HTO initiated by γ-radiation leads to a 1:1 mixture of labelled (+)- and *meso*-tartaric acids, while a mixture of labelled *meso*- and (±)-tartaric acids is obtained from *meso*-tartaric acid under these conditions[93]. The exchange between HTO and *chiro*-inositol, initiated by the intrinsic radiation, leads similarly to the formation of tritiated *chiro*-, *myo*-, and *allo*-inositols (but not *muco*-inositol)[95]. The greater ease of exchange of the equatorial hydrogen atoms compared with the axial atoms was noted in this instance.

3. Catalysis by Transition Metal Salts

From the point of view of the synthesis of labelled BAS, the preparative value of the last of the varieties of homogeneous catalysis considered here—isotope exchange with HTO in the presence of soluble chloroplatinates (Na_2PtCl_4 and K_2PtCl_4)—is low. This exchange also proceeds in an acid medium (acetic acid is added to homogenise the solution, while hydrochloric acid prevents the precipitation of metallic platinum) and requires an increased temperature (from 50° to 130 °C).[97–101] The mechanism of the exchange involves the formation of a complex of the type of a π-complex from the substrate and the transition metal[98]. The method described is therefore effective primarily for the hydrogen atoms of an aromatic ring, polynuclear compounds (napthalene, anthracene, and phenanthrene) exchanging specifically in the β-position. A comparative study[102] has shown that catalysis by K_2PtCl_4 is preferable (requires a lower temperature and a shorter time) than heterogeneous catalysis of the exchange with the solvent in the presence of platinum for the introduction of the deuterium label into hexoestrol, oestrone, testosterone, and cholesterol.

The homogeneous catalysis by mercury(II) chloride of the isotope exchange between a nucleoside [2-acetamido-6-chloro-9-(2′-deoxy-3′,5′-di-*O*-*p*-toluyl-D-ribofuranosyl)-9*H*-purine] and tritium-containing aromatic solvents—benzene and toluene (refluxing for 0.5 h)—has also been described[103]. Under these comparatively mild conditions, the efficiency of the exchange is low (a for the labelled nucleoside is smaller by a factor of approximately 10^4 than for the initial solvent) and the incorporated tritium is distributed between the purine and the carbohydrate moieties of the nucleoside molecule in proportions of 84 : 16.

IV. HETEROGENEOUS CATALYSIS OF HYDROGEN ISOTOPE EXCHANGE

The methods for the homogeneous catalysis of isotope exchange examined above make it possible to obtain tritium-containing compounds with a high value of a (of the order of several tens of c mmol^{-1} or at least several c mmol^{-1}) in only a few cases. In contrast to these procedures, in heterogeneous catalysis such high values of a can be obtained much more readily which constitutes the main advantage of this group of methods. In heterogeneous catalysis of isotope exchange in solution, both tritium-containing solvents (usually water) and gaseous T_2 can serve as sources of tritium.

1. Exchange with Tritium-Containing Solvents

In this case the experimental procedure usually consists in prolonged heating of a mixture of the test substance, the tritium-containing solvent, and the catalyst. The method has come to be widely employed since the 1950s and a compilation of early studies on this problem (up to 1964) can be found in the first edition of Evans's monograph[104]. The method is nowadays widely used and one of the largest centres of isotope production in the world—the Radiochemical Centre in Amersham—uses it for the industrial manufacture of a wide variety of tritium-labelled compounds, including a large number of BAS.

Table 1. Biologically active substances labelled by the method of heterogeneous catalytic exchange with the solvent (catalyst—Pt black)*.

Substance	a of solvent**	t, °C	Time, h	Yield, %	a of product, μc mmol^{-1}
	Solvent—HTO				
DL-Alanine	1.8	140	16	30	1 130
Anisomycin	150	100	16		285
DL-Aspartic acid	1.35	130	15	68	466
DL-Valine	1.08	120	16	80	20
Vitamin B_{12}	80	140	20	50	4 804
Glycine	1.04	100	8.5	58	176
DL-Glutamic acid	1.8	130	15	15	74
Guanine	70	145	20	50	150
Guanosine	90	130	16	20	481
Deoxyguanosine	87	120	3	0.2	1 000
Deoxycytidine	70	130	16	0.4	1 527
DL-3,4-Dihydroxyphenylalanine	1.35	125	16	35	268
DL-Isoleucine	1.8	155	16	40	314
Isonicotinoylhydrazine	100	125	16	72	1 323
Coumarin	6.3	130	16	34	2 242
DL-Leucine	0.54	120	66	47	103
5-Methylcytosine	100	130	16	10	15 600
Nicotine	35	100	72	33	82
Pyridoxin	200	120	16	3	1 500
DL-Serine	1.44	130	15	60	138
Stilboestrol	10	140	5	42	295
DL-Tyrosine	1.35	150	17	48	102
DL-Tryptophan	1.44	100	18	28	685
DL-Phenylalanine	2.16	140	16	50	1 800
	Solvent—70% AcOT				
Adenine	67	130	14	70	439
Adenosine	63	110	8	5	680
Androstenedione	40	150	48	50	3400
1,2-Benzanthracene	150	150	16	40	655
3,4-Benzopyrene	25	160	16	64	451
Hypoxanthine	50	150	15	30	630
Deoxyadenosine	67	100	6.5	7	627
Deoxyuridine	33	100	5	20	385
9,10-Dimethyl-1,2-benzanthracene	30	150	16	50	3250
Inosine	50	100	20	8	642
Corticosterone acetate	38	130	16	32	1590
Caffeine	25	150	16	36	1280
2-Methylcholanthrene	225	145	16	14	3150
Orotic acid	250	140	15	65	115
Prednisolone	160	150	5	10	685
Progesterone	88	140	16	43	509
Theophylline	75	140	16	21	1480
Thymidine	100	170	15	20	8600
Uracil	50	155	16	50	482
Uridine	75	120	16	35	1008
Cholesterol	180	155	16	23	1620
Cytosine	40	100	20	40	300
Oestradiol	200	140	16	21	174
Oestrone	14	130	90	56	188

*Reaction mixture—0.2–0.5 g of substance with 50–100 wt.% of catalyst in 2–4 ml of solvent.

**The values for the exchange with aminoacids and coumarin are quoted in c mmol^{-1}; in all other cases they are expressed in c ml^{-1}.

In the exchange with the solvent the catalysts can be virtually all Group VIII metals—platinum, palladium, cobalt, nickel, ruthenium, rhodium, and iridium—which are prepared by reducing their salts with $NaBH_4$ or by reducing their oxides with T_2 (or D_2) [105–108] and are used as such (platinum black, palladium black, etc.) or on various carriers (silica gel, activated charcoal, Al_2O_3, $BaCO_3$, $CaCO_3$). In Amersham preference is given to the platinum catalyst[105], but a high efficiency of the nickel (Raney nickel) and chrome-nickel catalysts has also been reported[109,110]. In choosing the catalyst, account must be taken of its influence on the intramolecular distribution of the added tritium (as a rule, platinum and palladium are less specific; for further details, see below) and also the fact that arsenic, sulphur, and certain nitrogen-containing compounds poison many (but not all) catalysts.

The most common medium in heterogeneous catalytic exchange in solution is HTO itself and also a 70 : 30 mixture of AcOT and HTO. In order to attain high values of a, a temperature of approximately 120–150 °C and a treatment time ranging from 2 to 20 h (in rare instances significantly longer—up to 65–90 h) are required. Under these conditions, tritium is introduced into the majority of low-molecular-weight BAS. The side reactions can be the migration of the Δ-bond (in unsaturated compounds), partial hydrolytic cleavage, and (when AcOT is used) acetylation, but the labelled side products have approximately the same value of a as the main compound. As a result, their separation does not as a rule present a specially difficult problem (which is an advantage over the Wilzbach method and its modifications). The activity a of the products naturally depends on the molar activity of the solvent and reaches up to 80% of the latter under most favourable conditions.

Table 1 presents specific results achieved by the Radiochemical Centre in the preparation by the method described (with platinum black as the catalyst) of certain low-molecular-weight BAS.[105] Evidently the ease of introduction of tritium into various compounds of even the same class (for example into aminoacids) is varied. In the majority of cases the exchange of the hydrogen in aromatic rings is easier than in aliphatic residues. One should also note that the values of a quoted in Table 1 for readily exchanging substances are by no means limiting values. Thus the synthesis of adenosine triphosphate with a = 23.3 c mmol^{-1} as a result of the platinum-catalysed exchange with HTO has been reported recently[21].

As regards the classes of BAS not mentioned in Table 1, the exchange with HTO in the presence of platinum (140 °C, 20–70 h) has been used to introduce tritium into terpenes (~2 μc mg^{-1});[111] the possibility of using the "self-activating" PtO_2 (HTO, 130 °C, 48 h) as a catalyst rather than platinum has been demonstrated for carbohydrates in relation to (−)-inositol (a = 3.39 μc mmol^{-1}).[112] However, Raney nickel, which catalyses the exchange between D_2O and α-methyl-D-mannopyranoside, β-methyl-D-galactopyranoside, 1,2-*O*-isopropylidene-α-glucofuranose, α,α-trehalose, melezitose, raffinose, and sucrose (100 °C, 10 h), is more promising for the introduction of a label into carbohydrates[109].

The principal disadvantages of the method under consideration are the use of very large amounts (up to 500 and more curies) of HTO and the relative severity of the exchange conditions. In order to overcome the first of these disadvantages, it has been suggested[113,114] that the labelled water required for the exchange be obtained by oxidising gaseous T_2 with a suspension of PtO_2 in AcOH, dioxan, or methanol in the apparatus where the exchange itself takes place; the exchange is catalysed by platinum formed on reduction of PtO_2, which makes it possible to obtain tritiated water with

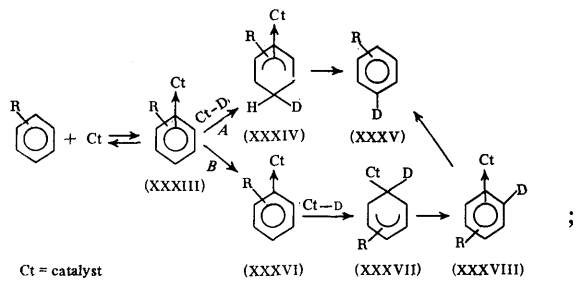
the maximum specific activity, to remove the excess T_2, and in many cases to eliminate the problem of the dissolution of the starting material. With the aid of this modification of the method, folic acid and uridine with $a \cong 1$–3 c mmol^{-1} were obtained (AcOH, 120 °C, 24 h),[113] while under milder conditions (dioxan or MeOH, ~20 °C, 4–24 h) geraniol, linalool, gibberelic acid, hydroxytetracyclin, and phenylalanine were synthesised with a ranging from 50 to 500 μc mmol^{-1}.[114]

The last example demonstrates that, as stated above, the attainment of a high value of a requires an increased temperature. This disadvantage of the method significantly restricts the range of BAS to which it can be applied, since biopolymers (particularly polypeptides and proteins) decompose on heating in most instances, while optically active aminoacids racemise. Thus, although L-phenylalanine exchanges with HTO at 30 °C in the presence of platinum without racemisation (since the side chain is not involved and more than 90% of the incorporated tritium is localised in the benzene ring), the overall degree of incorporation of tritium is small, while at 130 °C generally labelled DL-phenylalanine is obtained[115]. A high degree of racemisation (20–50% of the D-form) under the conditions of the exchange with HTO at 120–150 °C has also been noted[105] for L-tyrosine, L-tryptophan, and L-methionine, while the degree of racemisation in the case of arginine, lysine, and leucine did not exceed 5% and the values of a were below 200 μc mmol^{-1}.

In addition it has been shown with the aid of deuterium that the hydrogen isotope exchange involving the aromatic ring and catalysed by platinum under mild conditions is observed for phenylalanine and its tripeptide, but hardly occurs for the phenylalanyl and tyrosyl residues in proteins such as lysozyme and ribonuclease (the possible causes are steric hindrance to the adsorption of these residues on the catalyst or irreversible poisoning of the catalyst by the protein)[116]. Nevertheless, it is most likely that conformational factors play a decisive role, because, after prolonged treatment (40 °C, 72 h) with 35 c HTO and platinum black, it was possible to obtain labelled ribonuclease with $a = 17.3$ c mmol^{-1}; the bulk of the tritium was present in the valine, lysine, arginine, and glutamic acid residues[117]. Under the same conditions, tritium was introduced into oxidised ribonuclease much less effectively ($a = 0.2$ c mmol^{-1})—mainly into histidine and sulphur-containing and aromatic aminoacid residues. Tritium-containing polypeptides—ochratoxins A, B, and C (yield 80%, a up to 3 c mmol^{-1})—have been obtained[118] with the aid of heterogeneous catalytic exchange with the solvent (a mixture of HTO and AcOT, platinum black, 80 °C), ochratoxin A being labelled mainly (to an extent greater than 80%) in the phenylalanyl residue[118].

One of the slight disadvantages of the method is the uncertainty in the predicted location of tritium in the labelled compound obtained. These compounds are referred to as generally labelled or indefinitely labelled (in contrast to specifically labelled and uniformly labelled). The intramolecular distribution of tritium depends on a number of factors (primarily on temperature and on the nature of the catalyst) and it has been stated[119] that the reproducibility of the results of studies of the intramolecular distribution are poorly reproducible. The influence of temperature on the localisation of tritium in phenylalanine was noted above but even in high-temperature exchange (135 °C, 18 h), accompanied by complete racemisation, tritium is not distributed uniformly between the side chain and the aromatic ring, but is concentrated mainly in the latter (in proportions of 27 : 73).[120] A definite selectivity also obtains in a number of other instances: in catalysis by platinum black uracil exchanges with HTO mainly the hydrogen atoms in the 5- and 6-positions[121]; the exchange between (−)-inositol and HTO in the presence of PtO_2 leads to preferential (to the extent of 92.5%) inclusion of tritium in the 1- and 6-positions[117]; the exchange of sugars with D_2O in the presence of nickel takes place at $C_{(5)}$ and $C_{(6)}$ much faster than at $C_{(2)}$ and $C_{(3)}$;[109] cobalt catalyses the exchange of hydrogen in heterocycles exclusively in the α-position[108]. Poisoning of the catalyst also influences the location of tritium: thus, when pyridine is added, the exchange between HTO or D_2O and alkylbenzenes catalysed by Pd/C is selectively inhibited in the *ortho*-position relative to the alkyl group[122].

As regards the mechanism of the heterogeneous catalysis of the exchange with the solvent, it has been found that the exchange on different catalysts takes place in different ways. In the most common exchange of aromatic compounds in the presence of platinum it has been suggested[123] (on the basis of the results of a study of deuterium exchange) that a π-complex (XXXIII) is formed in the first stage. The complex then either interacts with the deuterium (tritium) sorbed on the catalyst (as a result of the dissociative chemisorption of water), being converted via a transition state (XXXIV) into the labelled compound (XXXV) ("associative mechanism"; A) or ("dissociative mechanism"; B) is first converted into a σ-complex (XXXVI), which reacts slowly with the deuterium (tritium) sorbed on the catalyst to form the addition product (XXXVII). The labelled compound (XXXV) is obtained from the latter via the labelled π-complex (XXXVIII). The dissociative mechanism is believed to be more probable[123–125]:



2. Exchange with Gaseous Tritium

The use of gaseous T_2 as a source of tritium in the heterogeneous catalysis of hydrogen isotope exchange in solution has a number of advantages over the use of tritium-containing solvents (see reviews[105,126]). These include primarily the possibility of carrying out the exchange at the usual temperature and of obtaining over a period of only several hours labelled compounds with $a \cong 1$–10 c mmol^{-1} (and sometimes even higher). The method consists in stirring a solution of 10–50 mg of the test substance in several millilitres of a suitable solvent in an atmosphere of 10–25 c T_2 in the presence of a catalyst. The overall radioactivity in the experiment is thus approximately ten times lower than in the exchange with a solvent. The exchange is faster if the pressure of T_2 is equal to the atmospheric pressure, but this condition is not obligatory and sometimes high values of a are attained in several hours even at a pressure of 160 mmHg.[127] The reaction is carried out in a special vacuum system (for a detailed description of such vacuum apparatus tested in practice, see Mikhailov et al.[128]).

The influence of various factors (solvent, temperature, and catalyst) on the exchange with T_2 has been inadequately investigated, but it has been established that there are apparently no special limitations as regards the choice of the solvent. Both organic solvents (dioxan, methanol,

hexane, CCl_4, CS_2, acetic acid, etc.)[129,130] and water or aqueous buffered solutions at neutral or alkaline pH are used[131]. The isotope exchange between T_2 and organic solvents is slight under these conditions; it increases slowly in the series dioxan < tetrahydrofuran < ethyl acetate[132], but even ethyl acetate can be used successfully (for example, in the exchange between T_2 and cholesterol)[133]. Satisfactory results are obtained even at 20–25 °C, but there is evidence[130,134] that an increase of temperature to 60–65 °C makes it possible to increase a of the exchange products by a factor of 2–3.

Palladium and platinum or their oxides (in the latter case the exchange is preceded by the reduction of the oxides to the metals) are most frequently used as catalysts[131,134] either as such or deposited on various carriers ($CaCO_3$, $BaSO_4$, activated charcoal, and Al_2O_3; in addition nickel[135], Rh/Al_2O_3,[136] and certain other catalysts are also employed). The nature of the carrier is not important[137,138], although in certain cases the use of $CaCO_3$ suppressed side reactions[138]. The method of activation of the catalyst is more important: in the preliminary reduction of PdO or PtO_2 by H_2 (instead of T_2) the value of a of the products decreases by a factor of 30–60.[129,134]

Table 2. Compounds labelled with the aid of heterogenous catalytic exchange with T_2 in solution[131] (catalyst—$PdO/BaSO_4$)*.

Compound	pH of solution	Time, h	Yield, μc	a, c mmol^{-1}
Adenosine	8.2	2	660	11.2
Adenosine monophosphate	10	16	295	—
Adenosine cyclic 3',5'-diphosphate**	10	16	290	28.3
Adenosine triphosphate	8	16	84	13.0
Deoxyadenosine triphosphate	10	16	250	9.6
Inosine monophosphate	8	0,33	195	4.9
Guanosine triphosphate	8.2	0,5	28	1.6
Puromycin	8	0,5	28	3.75

*10–30 mg of substance with a fourfold–fivefold excess (by weight) of catalyst in 1.5–2.5 ml of phosphate buffer (atmosphere of 10 c T_2).
**In an atmosphere of 25 c T_2.

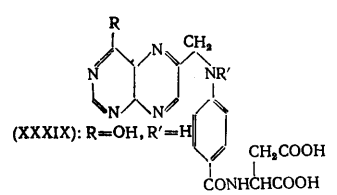
Heterogeneous exchange with T_2 in solution has been used successfully to introduce the label into carbohydrates, nucleotides, steroids, and certain other BAS containing aromatic or heteroaromatic rings and in most cases the selectivity (or even specificity) of the exchange was noted. D-glucose-1-*t* is obtained very readily (~20 °C, 1 h) as a result of the exchange with T_2 in 0.1 M phosphate buffer in the presence of $PdO/BaSO_4$ (a = 5.4 c mmol^{-1}).[131] Other aldoses—L- and D-arabinoses, L- and D-fucoses, D-ribose, maltose, and lactose—exchange just as specifically. The presence of the aldehyde group is an obligatory condition for the exchange: under analogous conditions, it is not possible to introduce tritium into sorbitol, α- and β-methyl glucosides, or trehalose[131]. Tritium has been introduced by the above method also into more complex compounds containing carbohydrate residues: platinum catalyses the exchange of T_2 with the aminoglycoside antibiotic sagamicin[139] and 30% Pd/C catalyses the exchange with heparin [water–ethylcarbitol (monoethyl ether of diethylene glycol), ~20 °C, 48 h];[140] nucleosides and nucleotides as well as the antibiotic puromycin with a high value of a and a radiochemical purity of 90–98% have been obtained by exchange in the presence of $PdO/BaSO_4$ in phosphate buffer (Table 2).[131]

Hydrogen in the 8-position exchanges most readily in purine derivatives[131]; among pyrimidines, tritium is introduced selectively in the 6-position in 5-hydroxyuracil and 5-aminouracil (5% $Pd/BaSO_4$, water, 23 °C, 3 h) with a = 0.35 and 2.36 c mmol^{-1} respectively[127].

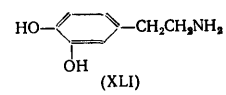
It is noteworthy that tritium introduced into galactosylceramide and glucosylceramide (10 c T_2, 10% Pd/C, on chloroform–methanol in the presence of pyridine, ~20 °C) is almost fully localised (to an extent greater than 98%) in the sphingolipid part of the molecule and of that more than 80% is in the aliphatic acid residue[141].

The exchange of T_2 with steroids—cholesterol (a up to 1 c mmol^{-1})[133,142] and oestrogens (oestrone, oestriol, and oestradiol; $a \cong 1.5$ c mmol^{-1})[134,143]—is catalysed by palladium or platinum, best results being obtained using benzoates in organic solvents (ethyl acetate or dioxan) with subsequent hydrolysis.

Among aminoacids, L-phenylalanine exchanges very readily with T_2 in solution in the presence of PtO_2 [134] or $PdO/BaSO_4$ [131]. In the latter case (phosphate buffer, pH 7, 20 °C, 1 h) a > 1 c mmol^{-1} was attained. The exchange is not accompanied by racemisation and involves almost exclusively (to an extent greater than 95%) the benzyl CH_2 group (the 3-position). Under analogous conditions, it was possible to obtain labelled L-alanine with a equal to only 10 μc mmol^{-1}.[131] This example illustrates strikingly the extreme ease of the exchange of the benzyl hydrogen atoms in the α-position, which has been confirmed also in other cases[129] and has been used to introduce tritium into a series of BAS containing benzyl or analogous groups. These compounds include, for example, folic acid (XXXIX), in which more than 60% of the incorporated tritium is localised in the 9-position after exchange with T_2 (10% Pd/CaO_3, 4 *N* KOH, ~20 °C, 1 h, a = 30–36 c mmol^{-1}), and 2-(3,4-dihydroxyphenyl)ethylamine (dopamine) (XLI), which belongs to the class of catecholamines, where more than 90% of the tritium label is introduced into the 2-position of the side chain after exchange with T_2 ($PdO/BaSO_4$, phosphate buffer, ~20 °C, 2 h, $a \cong 1$ c mmol^{-1}).[131] It is of interest to note that a compound related to folic acid—the medicinal preparation methotrexate (XL)—contains only 30% of the incorporated tritium in the 9-position (the conditions of the exchange were the same as for folic acid), evidently due to steric hindrance caused by the neighbouring methyl group[144]:



(XL): R=NH₂, R′=Me.



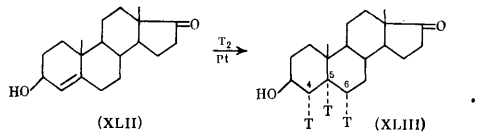
(XLI)

5-(3-Methylaminopropyl)- and 5-(3-dimethylaminopropyl)-10,11-dihydro-5*H*-dibenzo[*d*,*f*]azepins (the medicinal preparations desipramin and imipramin) as well as 5-(3-methylaminopropylidene)- and 5-(3-dimethylaminopropylidene-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclopentenes (the medicinal preparations nortriptyline and amitriptyline), which exhibit a powerful antidepressant activity, exchange with T_2 (PdO, methanol, ~20 °C, 45 min, a = 45, 15, 2, and 1.7 c mmol^{-1} respectively) almost exclusively at the methylene group located between the two aromatic rings[145]. Among other labelled medicinal substances obtained by exchange with T_2 and

exhibiting a high value of a, mention should be made of DL-propranol (PtdO, dioxan, 4.5 h, yields 37%, $a = 27.3$ c $mmol^{-1}$)[146] and 1-(1-phenylcyclohexyl)piperidine (the medicinal preparation phencyclidine) (PdO, methanol, yield 25%, $a = 4$ c $mmol^{-1}$).[147]

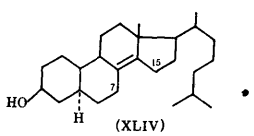
The number of biologically active substances which can be selected for the introduction of the tritium label by exchange with T_2 in solution is limited by the side reduction of certain groups and by the saturation of multiple bonds. Thus the attempt to introduce tritium into uridine by the above method ($PdO/BaSO_4$, phosphate buffer, 1 h) led only to dihydrouridine-5,6-*t*.[131] However these limitations are not absolute: for example, the exchange of aldoses mentioned above is not accompanied by the reduction of the aldehyde group. As regards unsaturated compounds, the saturation and isotope exchange of the latter proceed competitively and depend markedly on the conditions (catalyst and temperature). Thus the activation energy for the exchange involving cyclohexene in the presence of $Rh(PPh_3)_3Cl$ (homogeneous catalyst) is higher than for saturation and the latter is the main process at 30 °C, but at 60 °C the exchange proceeds more readily[148].

In the isotope exchange between T_2 and tricyclic antidepressants (see above) in the presence of palladium (from PdO) the degree of retention of the double bond is much greater than in the presence of PtO_2 or $PdO/BaSO_4$. Even better results are achieved when the stages involving the reduction of PdO to palladium and the exchange with antidepressants are separated and are carried out consecutively[145]. In the platinum-catalysed reduction of 3β-hydroxyandrost-4-en-17-one (XLII) by tritium the saturation and isotope exchange also proceeds simultaneously: in the labelled 3β-hydroxyandrostan-17-one (XLIII) obtained the tritium is distributed between the 4α-, 5α-, and 6α-positions in the proportions 37 : 43 : 20:[149]



(XLII) (XLIII)

As in heterogeneous catalysis of the exchange with the solvent, the mechanism of the exchange with gaseous T_2 (or D_2) is different for different catalysts (see, for example, Rudakov et al.[150]). In particular, for substituted aromatic compounds, chemisorption on the surface of the platinum or palladium catalysts in the form of π-complexes has been postulated[151,152], while in the case of the nickel catalyst the formation of σ,π-complexes is more likely[135]. The mechanism of the exchange with T_2 in aqueous media cannot be regarded as finally established; it has only been demonstrated that the substrate exchanges directly with T_2 and not with water[131]. In the discussion of the ionic and radical versions of this mechanism preference was given to the latter[131], which presupposes the homolysis of the C–H bond in the substrate RH in the first stage with formation of an intermediate free radical, adsorbed on the catalyst surface. This radical then interacts with the T atoms obtained as a result of the dissociation of T_2 on the catalyst, which leads to incorporation of the tritium in the substrate. This mechanism agrees well with, for example, the finding that cholest-8(14)-en-3-ol (XLIV) exchanges with D_2 (Pd catalyst, alcohol) the allyl hydrogen atoms at $C_{(7)}$ and and $C_{(15)}$. Evidently these most mobile atoms are split off with formation of an intermediate π-allyl complex[152]:



(XLIV)

V. CATALYTIC DEHALOGENATION

The method used for catalytic dehalogenation (stirring of the solution of the test substance in an atmosphere of gaseous T_2 in the presence of the catalyst) is identical with the method for heterogeneous exchange with T_2 (and also with that for catalytic reduction by T_2); in essence, catalytic dehalogenation differs from exchange by the fact that the T atom replaces a halogen atom rather than a hydrogen atom in the substrate molecule. We thus have the chemical reaction $RX + T_2 \rightarrow RT + TX$ and catalytic dehalogenation is accordingly one of the synthetic methods for the preparation of tritium-containing compounds. However, as already stated above, the inclusion of this method in the present review is justified, firstly, by the fact that it is most general for the synthesis of labelled BAS of different classes, and, secondly, because of its evident theoretical and practical similarity to exchange methods.

The advantages of the catalytic dehalogenation method are fairly clear. In the first place it makes it possible to obtain compounds with a high value of a and in many cases close to the theoretical value (29 c $mmol^{-1}$) corresponding to the complete substitution of one halogen atom by tritium. It is very significant that catalytic dehalogenation proceeds under very mild conditions (even at temperatures approximately in the range 0–5 °C and at a T_2 pressure of approximately 150–200 mmHg) and goes almost to completion in several hours. This can be explained by the greater thermodynamic ease of substitution of a halogen by T compared with substitution of H by T and by the fact that, owing to the smaller carbon–halogen bond dissociation energies compared with the C–H dissociation energies, the activation energy for dehalogenation is lower than for the isotope exchange of H for T. An important advantage is also the specificity of the introduction of the tritium label, determined by the position of the halogen in the molecule of the initial halogen-containing compound. However one should note the side reactions—isotope exchange and saturation of multiple bonds—can infringe the specificity and in certain cases (which are admittedly very few) the infringement can be very considerable[153–155].

The formation, together with the labelled compound, of tritiated hydrogen halides TX, which are catalytic poisons, can be included among the disadvantages of the method. In order to bind these poisons, KOH, NaOH, NH_3, Et_3N or $BaCO_3$ are added to the solution, which also displaces the equilibrium, accelerates the dehalogenation, and suppresses side reactions. The necessary initial halogen-substituted compounds are in most cases fairly readily available.

As already mentioned, the experimental conditions for catalytic dehalogenation are almost the same as in the isotope exchange with T_2. The amounts of T_2 used vary from several curies at pressures ranging from 100 to 760 mmHg. The range of solvents employed is very wide—aqueous alkalis, ethanol, methanol, dioxan, DMF etc. (water and alkaline agents enumerated above are added to the organic solvents when necessary).

Palladium (in the form of a black or on various carriers—activated charcoal, $CaCO_3$, $BaSO_4$, and Al_2O_3) is most often used as the catalyst; more rarely employed are platinum, nickel (Raney catalyst), and rhodium and more rarely still—osmium, iridium, and ruthenium. It is recommended that catalysts on carriers be employed, since this increases the active surface area and decreases the risk of poisoning of the catalyst. Satisfactory results are obtained when Pd/C and $Rh/CaCO_3$ or Rh/Al_2O_3 are used simultaneously for the dehalogenation of peptides and proteins[155–161]. The ease of substitution of a halogen by tritium decreases in the sequence I > Br > Cl > F in conformity with the increase of the carbon–halogen bond strength. It is therefore preferred to be-

with the corresponding iodides, particularly since even debromination sometimes proceeds unsatisfactorily[157], although, for example, 5-bromonicotinic acid is converted into labelled nicotinic acid in 94% yield (5% $Pd/CaCO_3$, tetrahydrofuran, in the presence of $BaCO_3$, 20 °C, 8 h).[162] However, in general the rate of reductive deiodination is several times greater than that of debromination (in the series of halogenobenzenes at approximately 20 °C, in the dark, and in the absence of a catalyst by a factor of 5.5) [163] and exceeds even more the rate of dechlorination and defluorination. Thus the homolytic dissociation of the carbon–halogen bond is the rate-limiting stage of the reaction and as a result of this it is in many instances possible to achieve the selective introduction of tritium into polyhalogeno-compounds[163–165].

Although catalytic dehalogenation has been used successfully for the introduction of the tritium label in virtually all classes of BAS, the method is most valuable in the preparation of labelled aminoacids, peptides, proteins, nucleotides, nucleosides, and nucleic bases. Catalytic dehalogenation is the main method for the synthesis of all aromatic aminoacids specifically labelled in the aromatic ring and having a high value of a (Table 3). In all cases dehalogenation is not accompanied by racemisation and leads to compounds with a high radiochemical purity. Hydrogenolysis in an atmosphere of pure T_2 yielded aminoacids with high values of a [for example, 14 c mmol^{-1} for L-tyrosine-3-t;[166,168] in other cases a mixture of T_2 and H_2 was used[167,170]].

Table 3. Labelled aminoacids obtained by catalytic dehalogenation.

Aminoacid	Initial halogen-containing compound	Catalyst	Medium	Chemical yield, %	Refs.
L-Phenylalanine-2,4-t_2	L-2,4-dibromophenylalanine	$Pd/CaCO_3$	aqueous ethanol	90–100	166
3,4-Dihydroxyphenylalanine-2,5,6-t_3	methyl ester of *O,O'*, *N*-triacetyl-2,5,6-tribromo-3,4-dihydroxyphenylalanine	Pd/C	dioxan (Et_3N)	54	167
3,4-Dihydroxyphenylalanine-2,5,6-t_3	2,5,6-tribromo-3,4-dihydroxyphenylalanine	$Pd/CaCO_3$	aqueous ethanol (KOH)	90–100	166
3,4-Dihydroxyphenylalanine-6-t	methyl ester of *O,O'*, *N*-triacetyl-6-bromo-3,4-dihydroxyphenylalanine	Pd/C	dioxan (Et_3N)	72	167
L-Tyrosine-3-t	3-iodo-L-tyrosine	10% Pd/C	aqueous methanol (KOH)	about 90	168
L-Tyrosine-3-t	3-iodo-L-tyrosine	$Pd/CaCO_3$	aqueous ethanol	90–100	166
L-Tyrosine-3,5-t	3,5-di-iodo-L-tyrosine	$Pd/CaCO_3$	aqueous ethanol	90–100	166
L-Tyrosine-3,5-t_2	3,5-di-iodo-L-tyrosine	Raney Ni	water (NaOH)	90–100	169
L-Tyrosine-3,5-t_2	3,5-di-iodo-L-tyrosine	Raney Ni	water (NaOH)	–	169
DL-Tryptophan-5-t	5-bromo-DL-tryptophan	10% Pd/C	water (KOH)	–	170
DL-Tryptophan-5-t	5-bromo-DL-tryptophan	$Pd/CaCO_3$	aqueous ethanol	90–100	166
DL-Tryptophan-4-t	4-chloro-DL-tryptophan	10% Pd/C	water (KOH)	–	170
L-Histidine-2,5-t_2	2,5-di-iodo-L-histidine	$Pd/CaCO_3$	aqueous ethanol (KOH)	–	166

The halogen can be substituted by tritium in the same way not only in the aromatic aminoacids themselves but also in their residues forming part of the composition of peptides and proteins. The interest of investigators was concentrated mainly on labelled peptide hormones (oxytocin, vasopressin, angiotensin (II), corticotropin, and α-melanotropin) and their analogues, for which values a of the order of several tens of c mmol^{-1} were attained. The results are presented in Table 4, which shows that the method is suitable for peptide molecules of different sizes (from 3 to 61 aminoacid residues). In all cases the dehalogenation was carried out at room temperature and almost all the labelled compounds of the peptide-protein type enumerated in Table 4 were obtained from iodine-containing precursors, i.e. peptides incorporating 3,5-di-iodotyrosine or 4-iodophenylalanine. The only exceptions are compounds Nos.3–6, which were synthesised from brominated precursors (containing 3,5-dibromotyrosine) or chlorinated precursors (containing 4-chlorophenylalanine). In one case (No.12) acetamido-D-alanyl(4-amino-3,5-di-iodo)-L-phenylalanine was dehalogenated and not the halogeno-analogue of the labelled peptide, the resulting tritium-containing dipeptide being converted into the labelled analogue of α-melanotropin by the usual methods of peptide synthesis.

The halogen is introduced into the initial halogenopeptides by various methods. Peptide synthesis makes it possible to obtain from a halogenoaminoacid halogenopeptides with a fixed position of the halogen, which is known exactly. The need for halogenopeptides of this kind stimulated the development of peptide syntheses from 3,5-di-iodo-L-tyrosine[178]. Another method consists in the treatment of the peptide (or of one of its synthetic precursors) with I_2 in a chloroform–methanol–water mixture (iodination of tyrosine residues in the 3- and 5-positions in the benzene ring)[176] or with iodine chloride, which iodinates the tyrosine ring or the histidine side chain depending on the conditions[159].

Catalytic dehalogenation is used very widely (particularly in recent times) for the synthesis of tritium-labelled nucleic bases, nucleosides, and their monophosphates. The label is introduced both into various positions of the nucleus of the base (usually with the aid of debromination) and into the methyl group (with the aid of dechlorination); for the compounds obtained, a varies in the range 5–25 c mmol^{-1} (Table 5).

Despite the fact that the conversion of the labelled bases into labelled nucleosides and their phosphates does not give rise to serious difficulties, it is frequently preferred to obtain the latter directly by the catalytic dehalogenation of the corresponding bromo-compounds. The same catalysts are used as a rule ($Pd/BaSO_4$, Pd/C) and the solvent usually consists of aqueous solutions of alkalis, the same values of a being attained (5–25 c mmol^{-1}). Adenosine-8-t,[179,188–190] guanosine-8-t,[189,190] inosine-8-t,[189] uridine-5-t,[179–181] 2-deoxyuridine-5-t,[179,180] cytidine-5-t,[181] adenosine monophosphate-8-t,[170,191,192] adenosine cyclic phosphate-8-t,[179,193] and uridine monophosphate-5-t [179] have been obtained in this way, but the yields did not exceed 50–60% owing to the reduced reactivity of the initial bromine-containing compounds. The latter is apparently determined by the existence in solution of a secondary structure stabilised by intramolecular forces[179]. The weakening of this stabilisation increases both the yield and the value of a of the labelled nucleosides and nucleoside phosphates obtained.

In order to convert the labelled monophosphates into labelled triphosphates, enzymatic phosphorylation is used. As in the case of peptides, the manufacture of tritium-containing nucleoside phosphates stimulated the development of methods for the synthesis of the monophosphates of the corresponding bromonucleosides[194].

Table 4. Labelled peptides and proteins obtained by catalytic dehalogenation.

No.	Substance	*n**	Labelled aminoacid residue	Catalyst	Medium	Yield, %	*a*, c mmol^{-1}	Refs.
1	Thyroliberin (releasing factor of thyrotropine)	3	histidine	Pd/Al_2O_3	phosphate buffer (pH 7.3)	–	60	171
2	Angiotensin II	8	tyrosine	5% $Pd/CaCO_3$	phosphate buffer	–	56.3	172
3	Asparagine^1-valine^5-angiotensin II	8	tyrosine-3,5-t_2	10% Pd/C	aqueous DMF (Et_3N)	–	2-3	173
4	Lysine^8-desglycineamide-vasopressin	8	tyrosine^2-3,5-t_2	Pd/Al_2O_3	acetic acid	–	–	174
5	Lysine^8-vasopressin	9	tyrosine^2-3,5-t_2	Pd/Al_2O_3	acetic acid	–	12	174
6	Lysine^8-vasopressin	9	phenylalanine^3-4-t	Pd/Al_2O_3	acetic acid	–	–	174
7	Lysine^8-vasopressin	9		10% Pd/Al_2O_3	phosphate buffer	–	10	175
8	Lysine^8-vasopressin	9	tyrosine^2-3,5-t_2	5% $Pd/CaCO_3$	aqueous methanol	–	25	176
9	Arginine^8-vasopressin	9	tyrosine-3,5-t_2	5% $Pd/CaCO_3$	aqueous methanol	–	17	176
10	Oxytocin	9	tyrosine-3,5-t_2	5% $Pd/CaCO_3$	methanol–ethyl acetate (1 : 1)	–	20	176
11	Oxytocin	9	tyrosine**	$Pd/CaCO_3$	phosphate buffer (pH 6.4)	–	35	177
12	D-alananine^1-4-azido-L-phenylalanine^7-norvalin^4-α-melanotropin	13	4-azidophenylalanine-3,5-t_2	Pd/C with $Rh/CaCO_3$	–	–	11	160
13	*N*-Bromoacetyl-D-alanine^1-glycine^3-norvaline^4-α-melanotropin	13	tyrosine^2-3,5-t_2	5% Pd/C with 5% $Rh/CaCO_3$	DMF	76	11-20	161
14	*N*-Diazoacetalglycine^1-norvaline^4-α-melanotropin	13	tyrosine^2-3,5-t_2	ditto	DMF	81	21-36	161
15	D-Serine^1-lysine^{17,18}-corticotropin-(1-18)-octadecapeptide	18	tyrosine^2-3,5-t_2	"	DMF	57	17	156
16	β-Corticotropin-(1-24)-tetracosapeptide (tetracosactrin, synacthen)	24	tyrosine^2-3,5-t_2	"	DMF	42	29	156
17	β-Corticotropin-(1-24)-tetracosapeptide	24	tyrosine^{23}-3,5-t_2	"	DMF	20	46	155
18	β-Corticotropin-(1-24)-tetracosapeptide	24	phenylalanine^7-4-t	"	DMF	18	27	157
19	Human corticotropin	39	tyrosine^{23}-3,5-t_2	"	DMF	30	25	158
20	α-Neurotoxin from *Naja nigricolis*	61	histidine	Pd-Rh/Al_2O_3	phosphate buffer (pH 7)		14	159

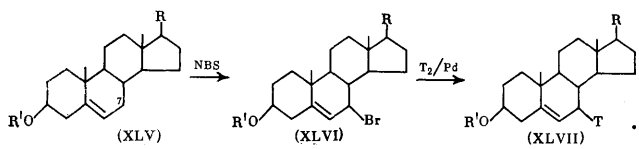
*Here *n* is the number of aminoacid residues.
**Tentatively.

Table 5. Labelled nucleic bases and their analogues obtained by catalytic dehalogenation.

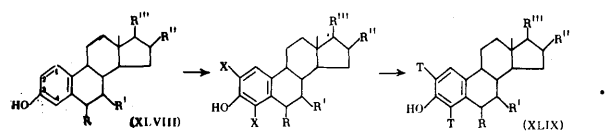
Labelled compound	Initial compound	Catalyst	Medium	Yield, %	*a*, c mmol^{-1}	Refs.
Uracil-5-*t*	5-bromouracil	$Pd/BaSO_4$	aqueous KOH	95-100	20-24	179
Uracil-5-*t*	5-iodouracil	Pt black			25	180
Uracil-5-*t*	5-iodouracil	5% $Pd/BaSO_4$	aqueous KOH	–	20	181
Uracil-6-*t*	6-bromo-2,4-dimethoxypyrimidine	5% $Pd/BaSO_4$	acetic anhydride	–	5-25	182
Cytosine-5-*t*	5-iodocytosine	5% $Pd/BaSO_4$	aqueous KOH	–	–	181
Thymine-methyl-*t*	5-(chloromethyl)uracil	10% $Pd/BaSO_4$	dioxan	70	15	154
Thymine-methyl-*t*	5-(hydroxymethyl)uracil	5% $Pd/BaSO_4$	aqueous acetic acid	77	15	154
5-Ethyluracil-4-*t*	4-chloro-5-ethyluracil	Pd/C	aqueous methanolic NaOH	85	–	183
5-Methylcytosine-methyl-*t*	5-(chloromethyl)-cytosine	PdO	dimethylacetamide	–	8	184
2,3-Dihydro-1,3-oxazine-2,5-dione-5-*t**	5-bromo-2,3-dihydro-1,3-oxazine-2,6-dione	5% $Pd/BaSO_4$	ethanol	64	11	185
Adenine-8-*t*	8-bromoadenine	Pd black	DMF	85-90	15	186, 187

*Synonym—3-oxauracil-5-*t*.

For steroids, the method of catalytic dehalogenation is particularly valuable, since it makes it possible to introduce the tritium label not only into a specific position and with retention of the Δ-bond, but also stereospecifically. Since steroids of type (XLV) are brominated by *N*-bromosuccinimide (NBS) exclusively in the 7α-position, the dehydrobromination of the resulting bromosteroids (XLVI) using Pd/C or $PdCaCO_3$ leads to 7α-labelled steroids (XLVII). Cholesterol-7α-*t*, pregnenolone-7α-*t*, and dehydroepiandrosterone-7α-*t* [195-198] were obtained by this method in yields exceeding 90% and with $a \cong 10$ c mmol^{-1}:[198]



Steroids (XLVIII) with an aromatic A ring, particularly the derivatives of oestradiol, are readily brominated (NBS) or iodinated (I_2 in aqueous methanol) in the 2- and 4-positions, which makes it possible to obtain subsequently by catalytic dehalogenation the steroid compounds (XLIX) labelled with tritium in these positions; oestradiol-2,4-t_2 (Raney Ni, aqueous NaOH),[169] oestriol-2,4-t_2 (Pd/C catalyst),[199] 16-oxo-oestradiol-2,4-t_2,[200] and isomeric oestra-1,3,5(10)-trienols-2,4-t_2 (5% Pd/Al_2O_3, ethyl acetate, $a = 15$–28 c mmol^{-1} [201]). The introduction of tritium into the 16-position in the 3α, 17α-dihydroxypregnane molecule has also been achieved by debromination (Pd/C catalyst):[202]



Vitamin K, labelled in the 6-position of the naphthoquinone nucleus (obtained from 6-bromo-2-methylnaphthaquinone with subsequent synthetic stages; (Pd/C, aqueous alcoholic NaOH, 5 °C), [203] α-tocopherol-(5-methyl-T) (obtained from 5-chloromethyl-α-tocopherol; Pd/C catalyst),[204] the antibiotic tetracycline-(7-T) (obtained from 7-chlorotetracycline, Pd/C, dioxan in the presence of tributylamine, $a = 5$ c mmol^{-1}),[205] the alkaloids papaverine (obtained from 6'-bromopapaverine; 10% Pd/C, dioxan containing Et_3N, $a = 43$ c mmol^{-1}; dehalogenation accompanied by exchange in methoxy-groups)[153] and *O*-benzoylecgonine-(benzoyl-2-T) [obtained from

O-(2-iodobenzoyl) ecgonine; Pd/C catalyst, $a = 21$ μc mg^{-1}],[206] the carcinogenic polycyclic aromatic hydrocarbon 7,8-dibenzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, indefinitely labelled in the pyrene nucleus (obtained from a mixture of the corresponding bromopyrenes; Pd black, dioxan containing Et_3N, $a = 1.25$ *c* mmol^{-1}),[207] *N*-(2-imidazolidinylidene)-2,6-dichloroaniline-4-*t*—the medicinal preparation clonidine exhibiting a hypotensive activity (obtained from the corresponding 4-bromo-derivative; Pd/C, tetrahydrofuran, $a = 1.6$ c mmol^{-1}),[165] and the cardiac preparations practol [practolol? (Ed.of Translation)] and isopractol [isopractolol? (Ed.of Translation)] (obtained from 3,5-dibromo-4-hydroxyacetanilide with subsequent synthetic stages; 10% Pd/C, methanol containing Et_3N, yield 86%, $a = 20$ c mmol^{-1})[208] should be mentioned as examples of the application of the method of catalytic dehalogenation for the synthesis of labelled BAS of other types.

In conclusion one should note that catalytic dehalogenation is not the only method for the replacement of a halogen by tritium. For this purpose, the following procedures have been recommended in recent years: reduction with sodium amalgam in HTO,[209] reduction with zinc and CH_3COOT in ether [in the conversion of 17α-bromopregnenolone into pregnenolone-17α-*t*],[210] reduction with Zn–Cu alloy in organic solvents in the presence of HTO (in the introduction of deuterium into the 3-*exo*-position of camphor starting from 3-bromocamphor),[211] electrochemical reduction in HTO [in the synthesis of uracil-5-*t* and deoxyuracil-5-*t* from the corresponding 5-bromo-derivatives][212,213], and finally simple heating of certain 5-iodopyrimidines (orotic acid, uracil, cytosine, and their derivatives) in dimethyl sulphoxide containing 5% HTO.[214] However, in all the instances enumerated the activity *a* of the resulting preparations was appreciably lower than in catalytic dehalogenation.

REFERENCES

1. E.A.Evans, "Tritium and Its Compounds" (Translated into Russian), Atomizdat, Moscow, 1970.
2. J.Filip and F.Vyšata, J.Labelled Compounds, **5**, 295 (1969).
3. J.Filip and F.Vyšata, Symposium, "New Methods of Obtaining Radioactive Preparations", Warsaw, 1969, p.325.
4. J.Filip and F.Vyšata, Radioisotopy, **10**, 93 (1969).
5. Czech.P.137 649 (1970); Chem.Abs., **75**, 110 330 (1971).
6. J.Moravek and J.Filip, Coll.Czech.Chem.Comm., **25**, 2697 (1960).
7. C.Parkanyi and F.Šorm, Coll.Czech.Chem.Comm., **28**, 2491 (1963).
8. J.W.Thanassi, J.Org.Chem., **36**, 3019 (1971).
9. L.A.Osterman, V.V.Adler, and R.Sh.Bibilashvili, Vop. Med.Khim., **13**, 200 (1967).
10. K.R.Shelton and J.M.Clark, Biochemistry, **6**, 2735 (1967).
11. K.R.Shelton and J.M.Clark, Biochem.Biophys.Res. Comm., **33**, 850 (1968).
12. U.Lauschke, E.Lodemann, and A.Wacker, J.Labelled Compounds, **7**, 441 (1971).
13. G.V.Sidorov and N.F.Masyoedov, Radiokhimiya, **16**, 922 (1974).
14. J.A.Elvidge, J.R.Jones, C.O'Brien, and E.A.Evans, Chem.Comm., 394 (1971).
15. R.N.Maslova, E.A.Lesnik, and Ya.M.Varshavsky, FEBS Letters, **49**, 181 (1974).
16. Ja.M.Varshavsky, E.A.Lesnik, and R.N.Maslova, "Prepr.Int.Symp.on Macromolecules", Helsinki, 1972, Vol.4, p.441.
17. R.G.Gamble, H.J.P.Schoemaker, E.Jekowsky, and P.R.Schimmel, Biochemistry, **15**, 2791 (1976).
18. H.J.P.Schoemaker, R.G.Gamble, G.P.Budzik, and P.R.Schimmel, Biochemistry, **15**, 2800 (1976).
19. R.N.Maslova, E.A.Lesnik, and Ya.M.Varshavskii, Mol.Biol., **9**, 310 (1975).
20. M.Tomasz, J.Olson, and C.M.Mercado, Biochemistry, **11**, 1235 (1972).
21. V.Valenta and J.Filip, Radioisotopy, **18**, 313 (1977).
22. D.Lichtenberg and F.Bergmann, J.Chem.Soc., Perkin Trans.I, 789 (1973).
23. M.Lajunen and H.Pilbacka, Acta Chem.Scand., **30A**, 391 (1976).
24. P.D.Klein and J.C.Knight, J.Amer.Chem.Soc., **87**, 2657 (1965).
25. J.C.Knight, P.D.Klein, and P.A.Szczepanik, J.Biol. Chem., **241**, 1502 (1966).
26. R.Bovara, R.Longhi, F.Nicotra, and G.Vecchio, J.Labelled Compounds, **13**, 425 (1977).
27. L.Szilágyi, P.Herzegh, and G.Bujtaš, Z.Naturforsch., **32B**, 296 (1977).
28. D.Muravskii, Symposium, "Organic Compounds Labelled with Radioactive Isotopes", Prague, 1977, p.120.
29. M.Levitz and J.Katz, Steroids, **5**. 11 (1965).
30. H.M.McIlhenny, J.Labelled Compounds, **12**, 523 (1976).
31. A.P.Tulloch, Lipids, **12**, 92 (1977).
32. L.D.C.Boc and H.J.van der Linde, Z.phys.Chem., **41**, 59 (1964).
33. G.Pattenden and R.Storer, J.Labelled Compounds, **12**, 551 (1976).
34. J.-C.Bonnafous, L.Fonzes, and M.Mousseron-Canet, Bull.Soc.chim.France, 4552 (1971).
35. D.J.Bennett, G.W.Kirby, and V.A.Moss, J.Chem.Soc. C, 2049 (1970).
36. G.A.Junk and H.J.Svec, J.Org.Chem., **29**, 944 (1964).
37. J.L.Morrison, Canad.J.Chem., **42**, 1009 (1964).
38. D.Balasubramanian, J.Amer.Chem.Soc., **89**, 5445 (1967).
39. G.Ronca and L.Willumsen, C.r.trav.Lbt.Carlsberg, **38**, 59 (1970).
40. C.Fini and A.Floridi, Ital.J.Biochem., **23**, 217 (1974).
41. R.Jeffries, J.Polymer Sci., **2A**, 5161 (1964).
42. I.M.Klotz and B.H.Frank, J.Amer.Chem.Soc., **86**, 3889 (1964).
43. Y.Kakuda, N.Perry, and D.D.Mueller, J.Amer.Chem. Soc., **93**, 5992 (1971).
44. M.S.Miller and I.M.Klotz, J.Amer.Chem.Soc., **95**, 5964 (1973).
45. M.S.E.Saleh and S.R.Srinavasan, Ann.chim., **59**, 1013 (1969).
46. N.N.Zatsepina and I.F.Tupitsyn, Symposium, "Organic Compounds Labelled with Radioactive Isotopes", Prague, 1977, p.195.
47. G.W.Kirby and L.Ogunkoya, J.Chem.Soc., 6914 (1965).
48. D.H.R.Barton, G.W.Kirby, W.Steglich, G.M.Thomas, A.R.Battersby, T.A.Dobson, and H.Ramuz, J.Chem. Soc., 2423 (1965).
49. D.H.R.Barton, D.S.Bhakuni, G.M.Chapman, and G.W.Kirby, J.Chem.Soc.C, 2134 (1967).
50. D.H.R.Barton, D.S.Bhakuni, G.M.Chapman, and G.W.Kirby, J.Chem.Soc.C, 1295 (1967).
51. K.Bloss, J.Labelled Compounds, **5**, 355 (1969).
52. N.Frank, Z.Naturforsch., **32B**, 240 (1977).
53. N.N.Zatespina, I.F.Tupitsyn, and L.S.Efros, Dokl. Akad.Nauk SSSR, **154**, 148 (1964).

54. B.C.Challis and E.M.Millar, J.Chem.Soc., Perkin Trans.II, 1111 (1972).
55. B.C.Challis and E.M.Millar, J.Chem.Soc., Perkin Trans.II, 1265 (1972).
56. L.Burrish, B.Tanacz, and J.Marton, Radioisotopy, **12**, 575 (1971).
57. P.M.Yavorsky and E.Gorin, J.Amer.Chem.Soc., **84**, 1071 (1962).
58. A.Kolodziejzyk and A.Arendt, Roczniki Chem., **51**, 659 (1977).
59. I.Ishiguro and B.Linzen, Z.physiol.Chem., **340**, 286 (1965).
60. G.W.Kirby, S.W.Shah, and M.Sandler, Chem.Comm., 819 (1967).
61. J.W.Daly and B.Witkop, J.Amer.Chem.Soc., **89**, 1032 (1967).
62. B.Bak, C.Dambmann, and F.Nicolaisen, Acta Chem. Scand., **21**, 1674 (1967).
63. R.S.Hsi, J.Labelled Compounds, **12**, 601 (1976).
64. J.L.Longbridge and F.A.Long, J.Amer.Chem.Soc., **89**, 1287 (1967).
65. J.L.Longbridge and F.A.Long, J.Amer.Chem.Soc., **89**, 1292 (1967).
66. B.C.Challis and E.M.Millar, J.Chem.Soc., Perkin Trans.II, 1618 (1972).
67. A.N.Sidorov, Optika i Spektrosk., **15**, 834 (1963).
68. J.B.Paine and D.Dolphin, J.Amer.Chem.Soc., **93**, 4080 (1971).
69. J.L.Garnett, B.Halpern, and R.S.Kenyon, Chem. Comm., 135 (1972).
70. G.G.Gusev, Yu.M.Kapustin, V.V.Ivanov, and M.M.Moskvina, Symposium, "Proizvodstvo Izotopov" (Manufacture of Isotopes), Atomizdat, 1973, p.165.
71. D.Kessel and M.Lubin, Biochemistry, **4**, 561 (1965).
72. A.T.Phillips and W.A.Wood, J.Biol.Chem., **240**, 4703 (1965).
73. A.A.Plentl and W.T.Kelly, Analyt.Biochem., **17**, 397 (1966).
74. M.C.Hochreiter and K.A.Schellenberg, J.Labelled Compounds, **5**, 270 (1969).
75. D.A.Upson and V.J.Hruby, J.Org.Chem., **42**, 2329 (1977).
76. A.Telc, B.Brunfelter, and T.Gosztonyi, J.Labelled Compounds, **8**, 13 (1972).
77. B.D.Hilton and R.D.O'Brien, J.Agr.Food Chem., **12**, 236 (1964).
78. N.Seiler, G.Werner, and K.-H.Schmidt, J.Labelled Compounds, **1**, 306 (1965).
79. U.Matsumoto, K.Shimada, M.Ikeda, I.Hayawaka, and Y.Nagase, Chem.Pharm.Bull.(Japan), **25**, 2195 (1977).
80. T.Gosztonyi, G.Bondesson, K.E.Domeij, and N.E.Stjernstrom, J.Labelled Compounds, **14**, 231 (1978).
81. T.Gosztonyi, J.Marton, and A.Kovacs, Nature, **208**, 381 (1965).
82. H.Emmerich and P.Schmialek, Z.Naturforsch., 21B, 855 (1966).
83. C.Mantescu, A.Genunche, and A.T.Balaban, J.Labelled Compounds, **2**, 261 (1966).
84. M.A.Long, J.L.Garnett, R.F.W.Wining, and T.A.Mole, J.Amer.Chem.Soc., **94**, 8632 (1972).
85. M.A.Long, J.L.Garnett, and R.F.W.Wining, J.Chem. Soc., Perkin Trans.II, 1298 (1975).
86. M.A.Long, J.L.Garnett, R.F.W.Wining, Tetrahedron Letters, 4531 (1976).
87. J.Seibles, D.M.Bollinger, and M.Orchin, Angew.Chem., **89**, 667 (1977).
88. M.G.Kuz'min, B.M.Uzhinov, G.Szent Györgyi, and I.V.Berezin, Zhur.Fiz.Khim., **41**, 769 (1967) [Russ. J.Phys.Chem., No.4 (1967)].
89. V.Gold, M.A.Major, and M.J.Gregory, J.Chem.Soc., Faraday Trans.I, 965 (1974).
90. V.Gold and M.A.Major, J.Chem.Soc., Faraday Trans.I, 977 (1974).
91. W.J.Spillane, Tetrahedron, **31**, 495 (1975).
92. M.Yoshida, H.Kaneko, A.Kitamura, T.Ito, K.Oohashi, N.Morikawa, H.Sakuragi, and K.Tokumaru, Bull. Chem.Soc.Japan, **49**, 1697 (1976).
93. M.Colossimo, J.P.Garvey, and V.Gold, J.Chem.Soc., Perkin Trans.II, 1595 (1975).
94. French P.7 439 686 (1975).
95. D.H.T.Fong, C.L.Bodkin, M.A.Long, and J.L.Garnett, Austral.J.Chem., **28**, 1981 (1975).
96. M.A.Long, J.L.Garnett, and C.P.Meakin, J.Labelled Compounds, **14**, 69 (1978).
97. J.L.Garnett and R.J.Hodges, Chem.Comm., 1001 (1967).
98. J.L.Garnett and R.J.Hodges, Chem.Comm., 1220 (1967).
99. J.L.Garnett and R.J.Hodges, J.Amer.Chem.Soc., **89**, 4546 (1967).
100. L.Blackett, V.Gold, and D.M.E.Reuben, J.Chem.Soc., Perkin Trans.II, 1869 (1974).
101. V.Gold, E.S.Gould, and D.M.E.Reuben, J.Chem.Soc., Perkin Trans.II, 1873 (1974).
102. J.L.Garnett, J.H.O'Keefe, and P.J.Claringbold, Tetrahedron Letters, 2687 (1968).
103. G.A.LePage and I.G.Junga, Canad.J.Chem., **43**, 1279 (1965).
104. E.A.Evans, "Tritium and Its Compounds" (Translated into Russian), Atomizdat, Moscow, 1970, p.103.
105. E.A.Evans, "Tritium and Its Compounds", Butterworths, London, 1974, p.300.
106. G.E.Galf, J.L.Garnett, and V.A.Pikles, Austral.J. Chem., **21**, 961 (1968).
107. G.E.Galf and J.L.Garnett, Austral.J.Chem., **21**, 1221 (1968).
108. G.E.Galf and J.L.Garnett, Chem.Comm., 306 (1967).
109. H.J.Koch and R.S.Stuart, Carbohyd.Res., **59**, C1 (1977).
110. V.A.Shalygin, Yu.N.Pisarev, and Ya.D.Zel'venskii, Symposium, "Proizvodstvo Izotopov" (Manufacture of Isotopes), Atomizdat, Moscow, 1973, p.190.
111. F.Schlegelmilch and H.Schildknecht, "Symposium über Zonenschmelzen und Kolonnenkristallisieren, Karlsruhe, 1963", p.437.
112. C.M.Fernandez and J.L.Garnett, J.Labelled Compounds, **3**, 155 (1967).
113. K.V.Viswanathan, O.M.G.Nair, R.Lakshmy, and A.S.Garana, "Proc.Symp.on Nuclear and Radiation Chemistry, Poona, 1967", p.280.
114. I.Pri-Bar and O.Buchman, Chem.Comm., 1631 (1970).
115. G.E.Galf, J.L.Garnett, B.H.Halpern, and K.Turnbull, Nature, **209**, 502 (1966).
116. R.S.Norton and J.H.Bradbury, J.Catalysis, **39**, 53 (1975).
117. M.Noyer, A.G.Schnek, and J.Leonis, Arch.Int.Physiol. et Biochem., **83**, 391 (1975).
118. F.C.Chang and F.S.Chu, J.Labelled Compounds, **12**, 231 (1975).
119. D.G.Crowter, E.A.Evans, and R.W.Lambert, Chem. Ind.(London), 899 (1960).
120. M.C.Clifford, E.A.Evans, A.E.Kilner, and D.C.Warrell, J.Labelled Compounds, **11**, 435 (1975).
121. T.Ito, M.Tatara, and Y.Kasida, Int.J.Radiat.Isotop., **24**, 81 (1973).

122. C.G.Macdonald and J.S.Shannon, Tetrahedron Letters, 1349 (1963).
123. J.L.Garnett and W.A.Sollich, J.Catalysis, **2**, 339 (1963).
124. J.L.Garnett and W.A.Sollich-Baumgartner, J.Phys. Chem., **68**, 3177 (1964).
125. J.L.Garnett and W.A.Sollich-Baumgartner, J.Phys. Chem., **69**, 3526 (1965).
126. M.Frangopol, Isotop.Radiation Technol., **9**, 447 (1972).
127. S.Noll, M.Kiessling, and K.-H.Heise, Isotopenpraxis, **13**, 324 (1977).
128. K.S.Mikhailov, O.V.Lavrov, and N.F.Myasoedov, Symposium, "Organicheskie Soedineniya Mechennye Radioaktivnymi Izotopami" (Organic Compounds Labelled with Radioactive Isotopes), Prague, 1977, p.253.
129. I.Pri-Bar and O.Buchman, Int.J.Appl.Radiation Isotopes, **27**, 53 (1976).
130. M.Angoso Marino and F.Kaiser Ruiz del Olmo, Junta Energ.Nucl.(Spain) (Rep.), 383 (1977); Chem.Abs., **88**, 190 261 (1978).
131. E.A.Evans, H.C.Sheppard, J.C.Turner, and D.C.Warrell, J.Labelled Compounds, **10**, 569 (1974).
132. J.Marton and A.Kovacs, Radioisotopy, **12**, 595 (1971).
133. J.Roemer, U.Preiss, and H.Wagner, Radiochim.Radioanal.Letters, **30**, 123 (1977).
134. O.Buchman, I.Pri-Bar, M.Shimoni, and L.Smoskowitz, J.Labelled Compounds, **10**, 345 (1974).
135. D.Zidarov, A.Andreyev, N.Neshev, and D.Shopov, Dokl.Bolg.Akad.Nauk, **29**, 845 (1976).
136. G.D.Kuehn, J.Labelled Compounds, **9**, 171 (1973).
137. R.Cardinaud and J.-C.Bouchet, Bull.Soc.chim.France, 834 (1965).
138. M.Guillaume, Bull.Soc.roy.sci.Liege, **36**, 696 (1967).
139. T.Deguchi, S.Okumura, A.Ishii, and M.Tanaka, J.Antibiot., **30**, 993 (1977).
140. G.H.Barlow and E.V.Cardinal, Proc.Soc.Exp.Biol. Med., **123**, 831 (1966).
141. A.Poulos and A.C.Pollard, J.Labelled Compounds, **14**, 17 (1978).
142. I.F.Tupitsyn, T.V.Bichul', V.I.Bernotas, G.G.Gusev, N.N.Zatsepina, B.A.Klimashevskaya, N.S.Pigoreva, G.P.Prokof'eva, and S.V.Chinyakov, Symposium, "Proizvodstvo Izotopov" (Manufacture of Isotopes), Atomizdat, Moscow, 1973, p.158.
143. J.H.Block and C.Djerassi, Steroids, **22**, 591 (1973).
144. S.F.Zakrzewski, E.A.Evans, and R.F.Phillips, Analyt. Biochem., **36**, 197 (1970).
145. O.Buchman and I.Pri-Bar, J.Labelled Compounds, **14**, 263 (1978).
146. O.Buchman, I.Pri-Bar, and Y.Hagag, J.Labelled Compounds, **10**, 519 (1974).
147. A.Kalir, D.Balderman, M.Torten, O.Buchman, and I.Pri-Bar, J.Labelled Compounds, **13**, 445 (1977).
148. A.L.Odell, J.B.Richardson, and W.R.Roper, J.Catalysis, **8**, 393 (1967).
149. Y.J.Abulhaji, J.Labelled Compounds, **7**, 33 (1971).
150. E.S.Rudakov, A.E.Shilov, and A.A.Shteinman, Symposium, "Rol' Koordinatsii v Katalize" (The Role of Coordination in Catalysis), Izd.Naukova Dumka, Kiev, 1976, p.142.
151. T.Meshi and Y.Sato, Bull.Chem.Soc.Japan, **37**, 683 (1964).
152. V.Ku, J.Palmer, S.Siegel, and R.Claugh, J.Catalysis, **44**, 449 (1976).
153. J.Devillers, M.Winand, and B.Bettens, J.Labelled Compounds, **12**, 219 (1976).
154. B.Bohaček and J.Filip, Symposium, "Organicheskie Soedineniya Mechennye Radioaktivnymi Izotopami" (Organic Compounds Labelled with Radioactive Isotopes), Prague, 1977, p.29.
155. D.E.Brundish and R.Wade, J.Chem.Soc., Perkin Trans.I, 2875 (1973).
156. D.E.Brundish, J.R.Martin, and R.Wade, J.Chem.Soc., Perkin Trans.I, 2182 (1976).
157. D.E.Brundish and R.Wade, J.Chem.Soc., Perkin Trans. I, 2186 (1976).
158. D.E.Brundish and R.Wade, Biochem.J., **165**, 169 (1977).
159. A.Menez, J.L.Morgat, P.Fromageot, A.-M.Ronseray, P.Boquet, and J.-P.Chaugeux, FEBS Letters, **17**, 333 (1971).
160. A.Eberle and R.Schwyzer, Helv.Chim.Acta, **59**, 2421 (1976).
161. A.Eberle, W.Hubscher, and R.Schwyzer, Helv.Chim. Acta, **60**, 2895 (1977).
162. B.R.Clark, J.Labelled Compounds, **12**, 535 (1976).
163. Y.Wu and H.J.Ache, J.Phys.Chem., **73**, 2424 (1969).
164. T.Hesselbo, Int.J.Appl.Radiat.Isotop., **16**, 329 (1965).
165. M.Stiasni and H.Stahle, J.Labelled Compounds, **14**, 51 (1978).
166. L.Birkofer and K.Hempel, Chem.Ber., **96**, 1373 (1963).
167. E.Schreier, W.Pacha, and J.Rutschmann, Helv.Chim. Acta, **46**, 954 (1963).
168. L.Carlsson and I.Sjoholm, Acta, Chem.Scand., **20**, 259 (1966).
169. J.Nunez, C.Jacquemin, and J.Roche, Int.J.Appl. Radiat.Isotop., **13**, 573 (1962).
170. W.Lovenberg, R.E.Bensinger, R.L.Jackson, and J.W.Daly, Analyt.Biochem., **43**, 269 (1971).
171. P.Pradelles, J.L.Morgat, P.Fromageot, C.Olivier, P.Jacquet, D.Gourdji, and A.Tixier-Vidal, FEBS Letters, **22**, 19 (1972).
172. J.L.Morgat, L.T.Hung, and P.Fromageot, Biochim. Biophys.Acta, **207**, 374 (1970).
173. Hung.P.13 924 (1977); Chem.Abs., **88**, 191 487 (1978).
174. D.Kery, I.Teplan, and I.Mezo, Symposium, "Organicheskie Soedineniya Mechennye Radioaktivnymi Izotopami" (Organic Compounds Labelled with Radioactive Isotopes), Prague, 1977, p.89.
175. P.Pradelles, J.L.Morgat, P.Fromageot, M.Camier, D.Bonne, P.Cohen, J.Bockaert, and S.Jard, FEBS Letters, **26**, 189 (1972).
176. G.Fluoret, S.Terada, F.Yang, S.H.Nakagawa, T.Nakahara, and O.Hechter, Biochemistry, **16**, 2119 (1977).
177. J.L.Morgat, L.T.Hung, R.Cardinaud, P.Fromageot, J.Bockaert, M.Imbert, and F.Morel, J.Labelled Compounds, **6**, 276 (1970).
178. K.Pawelczak, B.Rzeszotarska, and B.Nadolska, Annalen, 1521 (1976).
179. N.F.Myasoedov, G.N.Sidorov, A.F.Usatyi, and N.S.Marchenkov, Symposium, "Organicheskie Soedineniya, Mechennye Radioaktivnymi Izotopami" (Organic Compounds Labelled with Radioactive Isotopes), Prague, 1977, p.228.
180. J.Filip and H.Havliček, Jad.Energ., **15**, 388 (1969).
181. Czech.P.137 984 (1970); Chem.Abs., **76**, 25 545 (1972).
182. Czech.P.133 141 (1969); Chem.Abs., **73**, 77 276 (1970).
183. K.K.Gauri, K.-W.Pflughaupt, and R.Müller, Z.Naturforsch., **24B**, 833 (1969).
184. B.Černy and J.Hanuš, Symposium, "Organicheskie Soedineniya Mechennye Radioaktivnymi Izotopami" (Organic Compounds Labelled with Radioactive Isotopes), Prague, 1977, p.159.

185. J.Filip, J.Škoda, and J.Farkas, Symposium, "Organicheskie Soedineniya Mechennye Radioaktivnymi Izotopami" (Organic Compounds Labelled with Radioactive Isotopes), Prague, 1977, p.162.
186. J.Roemer and M.Kiessling, Isotopenpraxis, 11, 150 (1975).
187. M.Kiessling, Symposium, "Organicheskie Soedineniya Mechennye Radioaktivnymi Izotopami" (Organic Compounds Labelled wtih Radioactive Isotopes), Prague, 1977, p.202.
188. USSR P.249 391 (1972); Ref.Zhur.Khim., 8N351 (1973).
189. V.M.Vdovenko, V.N.Bobrova, E.G.Airapetyants, and A.V.Zharkov, Symposium, "Proizvodstvo Izotopov" (Manufacture of Isotopes), Atomizdat, Moscow, 1973, p.180.
190. J.Filip and L.Bohaček, Radioisotopy, 12, 949 (1971).
191. V.M.Vdovenko, V.N.Bobrova, L.S.Gordeeva, and V.K.Dedova, Radiokhimiya, 14, 457 (1972).
192. L.Bohaček and J.Filip, Radioisotopy, 14, 783 (1973).
193. I.I.Kolesnikova and L.A.Timofeeva, Symposium, "Issledovaniya v Oblasti Khimii i Tekhnologii Izotopov i Mechenykh Soedinenii" (Studies in the Chemistry and Technology of Isotopes and Labelled Compounds), Leningrad, 1977, p.3.
194. K.S.Mikhailov, N.L.Chichikina, V.S.Orlova, N.S.Marchenkov, and N.F.Myasoedov, Symposium, "Organicheskie Soedineniya Mechennye Radioaktivnymi Izotopami" (Organic Compounds Labelled with Radioactive Isotopes), Prague, 1977, p.111.
195. M.Gut and M.Uskoković, Naturwiss., **47**, 40 (1960).
196. M.Gut and M.Uskoković, J.Org.Chem., **25**, 792 (1962).
197. R.B.Clayton and A.M.Edwards, J.Biol.Chem., **238**, 1966 (1963).
198. V.S.Volkova, F.V.Tatarkina, L.I.Kaklyushkina, N.A.Ignat'eva, I.F.Tupitsyn, and Ts.I.Efimova, Symposium, "Organicheskie Soedineniya Mechennye Radioaktivnymi Izotopami" (Organic Compounds Labelled with Radioactive Isotopes), Prague, 1977, p.53.
199. B.H.Albrecht and D.D.Hagerman, Steroids, **19**, 177 (1972).
200. M.M.Coombs and H.R.Roderick, Steroids, **11**, 925 (1968).
201. A.D.Fraser, S.J.Clark, and H.H.Wotiz, J.Labelled Compounds, **12**, 213 (1976).
202. S.Weinman and J.Livet, J.Labelled Compounds, **4**, 344 (1968).
203. T.Konishi, S.Baba, and T.Matsuura, Radioisotopes, **20**, 665 (1971).
204. E.A.Evans and R.F.Phillips, J.Labelled Compounds, **5**, 12 (1969).
205. J.Hanuš, J.Kozel, and J.Beneš, Symposium, "Organicheskie Soedineniya Mechennye Radioaktivnymi Izotopami" (Organic Compounds Labelled with Radioactive Isotopes), Prague, 1977, p.73.
206. US P.4 071 522 (1978); Chem.Abs., **88**, 191 201 (1978).
207. D.J.McCaustland, D.L.Fischer, W.P.Duncan, E.J.Ogilvie, and J.T.Engel, J.Labelled Compounds, **12**, 583 (1976).
208. G.Stacher, M.Frez, S.Cohen, A.Cohen, and O.Buchman, J.Labelled Compounds, **13**, 59 (1977).
209. D.H.G.Grout and J.A.Corkill, Tetrahedron Letters, 4355 (1977).
210. L.Milewich and L.R.Axelrod, J.Labelled Compounds, **7**, 101 (1971).
211. L.M.Stephenson, R.V.Gemmer, and S.P.Current, J.Org.Chem., **42**, 212 (1977).
212. C.Bratu, J.Labelled Compounds, **7**, 161 (1971).
213. C.Bratu, Symposium, "Organicheskie Soedineniya Mechennye Radioaktivnymi Izotopami" (Organic Compounds Labelled with Radioactive Isotopes), Prague, 1977, p.37.
214. J.J.Kopecký and B.Pavlú, Coll.Czech.Chem.Comm., **37**, 2039 (1972).

Shemyakin Institute of
Bio-organic Chemistry,
USSR Academy of Sciences,
Moscow