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Review Article

Solid-phase reactions as approach to the synthesis of organic compounds labelled with tritium†

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Abstract: The solid-phase method for hydrogenolysis, hydrogenation and isotope exchange reactions is described. Its potential for tritium labelling of organic compounds including lipids, nucleotides, amino acids, peptides and pharmaceuticals is demonstrated. The influence of the reaction conditions on the yield and specific radioactivity of the labelled compounds is considered. It is shown that results can be interpreted by the dependence of tritium spillover reactions on the nature of the initial compound and other factors. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: solid-phase reactions; labelled; tritium; spillover

Introduction

The solid-phase method for conducting hydrogenolysis, hydrogenation and isotope exchange reactions is described in this paper. The peculiarity of such reactions is that they are conducted in solid phase without any solvent.[1] The scheme of solid-phase reactions is presented in Figure 1. According to this method, an organic substance is applied on a support (sorbent) where a metal catalyst has been applied preliminarily, and this system is heated under a tritium gas atmosphere. Solid-phase hydrogenolysis proved to be the process of choice for introducing a tritium label into organic compounds. Using these reactions, tritium-labelled lipids, peptides, proteins, nucleic acids, carbohydrates, glycoproteins, nucleoproteins, lipoproteins, glycolipids and many other classes of biologically active compounds have been obtained rather easily.

Plentiful data on the reactions of various compounds applied on a support with activated hydrogen species formed on the metal catalysts have been reported. The enhanced mobility of activated hydrogen on inert supports has been discovered.[2] The flow of activated hydrogen species from one phase to another has been called *hydrogen spillover*. It is hydrogen (tritium) spillover that determines the possibility of solid-phase hydrogenolysis. Data supporting both the atomic and protic natures of the spillover species can be found in the literature (Figure 1).

It is considered that the first step of spillover process is homolytic cleavage of the H–H bond on the metal catalyst, regardless of whether hydrogen atoms or protons participate in the spillover. The rate-limiting step is either the surface diffusion on the support or the transfer of particles from the catalyst to the support. Since the activation energies of these elementary steps are different, the limiting step of spillover may change upon temperature variation.[3] The prevalent opinion is that hydrogen spillover is limited by diffusion over the surface of the support.[4]

There is an opinion[5] that weakly chemisorbed hydrogen can be regarded as atomic, while strongly chemisorbed hydrogen can be taken as protons. Quantitative data obtained by spectroscopic methods do not allow one to identify unequivocally the nature of activated species in the solid-phase catalytic reactions. Hence, it can be proposed that tritium is also able to react with a substrate in either atomic or ionic form as shown in Figure 1.

Noticeable participation of the atomic tritium in hydrogenolysis or exchange reactions is expected only at elevated temperatures that are not used for labelling of biologically active compounds. The reason is that the reaction of radical elimination is able to compete with a more easy process of the atomic tritium recombination.

A mathematical model of spillover has been considered in several publications.[6–8] This model assumes

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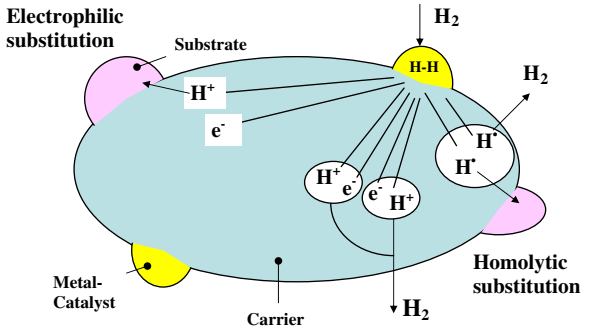

Figure 1 Scheme of solid-phase hydrogenolysis reaction.[2] (This figure is available in colour online at www.interscience.wiley.com/journal/jlcr.)

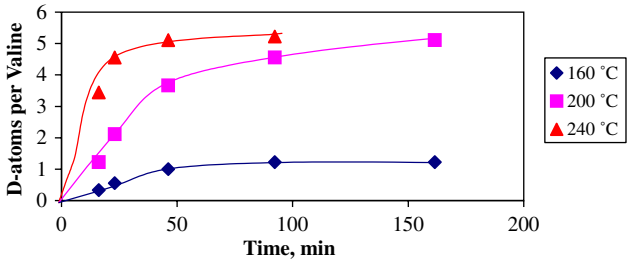

Figure 2 Kinetics of deuterium incorporation to L-valine at various temperatures. (This figure is available in colour online at www.interscience.wiley.com/journal/jlcr.)

that the activated species are formed on the source metal and the support conveys these species to the substrate and back. One-, two- and three-dimensional diffusion kinetic models for hydrogenolysis of a supported organic substrate have been constructed.[8] The models take into account the concentration gradient of the spillover hydrogen and poisoning of the catalyst during the reaction. It was shown that hydrogenation is almost localized in the reaction areas around the crystallites of the metal catalyst; hence, there exists some ratio of the catalyst, the support and the substrate optimal for this particular reaction.

Solid-phase reactions in the synthesis of tritium-labelled preparations

Solid-phase isotope exchange

Isotope exchange is the method of choice for introducing a tritium label, as it does not require the synthesis of precursors. In addition, isotope exchange allows one to prepare a labelled specimen using very small amounts (5–10 mg) of biologically active substances, which are often scarce and expensive.

The efficiency of solid-phase isotope exchange depends appreciably on the melting point of the organic compound. In view of this fact, all compounds can be divided into two groups. The first one includes substances that behave like liquids at the chosen reaction temperature (in this case, the molecules can migrate over the support and with respect to one another); the second group comprises compounds that exist as crystals during the reaction (in this case, direct contact with the catalyst active sites is impossible and the degree of isotope exchange mainly depends on the efficiency of tritium spillover). Naturally, the regularities of insertion of the label into compounds of the first and second groups are dissimilar.

On label introducing into L-valine (Figure 2) and L-alanine (Table 1) it was shown that the degree of isotope exchange smoothly increases with the temperature and the kinetic curves reach a plateau. [9,10]

A similar situation is observed in the introduction of the label into adenine, guanine, xanthine, hypoxanthine, deazaguanine (Tables 2–4) and 3-indolylacetic acid (Figure 3).

The curves obtained for low-melting compounds of lipid nature look quite different (Tables 5 and 6 and Figures 4 and 5).

Tables 5 and 6 and Figures 4 and 5 illustrate that kinetic curves at different temperatures and temperature dependences of RMR have a clear maximum for catalysts that are considerably different in activity. The appearance of maxima on these dependences can be explained in the following way. The molecules of, for example, a low-melting fatty acid, unlike those of high-melting compounds, can create a certain structured system around the active sites of the catalyst[15]; a layer of chemisorbed molecules functioning as ligands appears on the catalyst surface.[16,17] These systems are more stable against poisoning and deactivation. Therefore, the rate and the degree of exchange during labelling of such substances are higher than those in the case of high-melting compounds.[14,15]

As the temperature and process duration increase, the degree of isotope exchange becomes higher and the rate of side processes also increases. Starting from certain temperatures, the structured chemisorbed layer is destroyed; this stops the isotope exchange and induces decomposition of, first, the fraction of the specimen with the highest degree of labelling.[15] This accounts for the appearance of maxima in the plots for the dependence of the molar radioactivity of fatty acids on the temperature and the duration of isotope exchange.[14,18]

A study of the dependence of the degree of labelling on the length of the carbon chain in homologues of fatty acids whose melting points are markedly lower than

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Table 1 Dependence of specific activity of L-alanine on temperature and reaction time[10]

Method (I)			Method (II)		
Temperature (°C)	Time (min)	Sp. act (PBq/mol)	Temperature (°C)	Time (min)	Sp. act (PBq/mol)
70	90	0.044	130	60	0.296
90	40	0.068	150	40	0.444
110	20	0.096	170	30	0.852
130	20	0.263	190	20	1.148
155	20	0.778			

Method (I): L-alanine was sorbed on charcoal (Norit A, Serva, substrate-to-carrier ratio of 1:50), mixed with 5% Rh/Al_2O_3 (25:10) and heated with tritium gas. Method (II): Solution of L-alanine and $RhCl_3$ (2:1) in water added to 5% $Pd/CaCO_3$ (10:1) and dried by lyophilization. The resultant mixture was heated with tritium gas.

Table 2 Relative molar radioactivity and yield of adenine and guanine (5% $Pd/CaCO_3$, τ= 60 min) at various temperatures[11]

Temperature (°C)	Adenine		Guanine	
	Yield (%)	RMR (%)	Yield (%)	RMR (%)
20	89	1.4	89	1.1
120	91	1.8	81	1.4
140	87	16.8	77	5.3
150	78	34.7	73	13.7
160	79	54.7	69	21.1
180	84	100	65	59.0
200	—	—	69	74.7
220	—	—	75	82.1

Note: Here and in other tables and figures, τ represents duration of the experiment and RMR the relative molar radioactivity; the highest molar radioactivity attained in this series of experiments was taken to be 100% .

Table 3 Relative molar radioactivity and yield of xanthine and hypoxanthine (5% $Pd/CaCO_3$, τ= 60 min) at various temperatures[11]

Temperature (°C)	Xanthine		Hypoxanthine	
	Yield (%)	RMR (%)	Yield (%)	RMR (%)
20	81	0.8	88	1.6
120	83	2.4	—	—
140	72	20.9	81	12.8
150	78	50.0	—	—
160	78	67.4	84	16.3
180	71	96.5	68	61.6
200	70	100	64	97.7
220	—	—	81	96.5

the temperature at which the isotope exchange is carried out showed a linear decrease in the molar radioactivity with a decrease in the chain length (Figure 6). Hence, the isotope exchange for this type of substance is a probability process.[14,19]

However, for high-melting sodium salts of acetic, propionic and butyric acids, the molar radioactivity did not show this type of unambiguous dependence on the number of carbon atoms (Figure 7).[20]

Table 4 Relative molar radioactivity and yield of deazaguanine (5% $Pd/CaCO_3$–substrate ratio is 10:1, τ= 15 min) at various temperatures[12]

Temperature (°C)	Yield (%)	RMR (%)
140	69	3
160	65	15
180	62	62
200	61	86
220	57	100

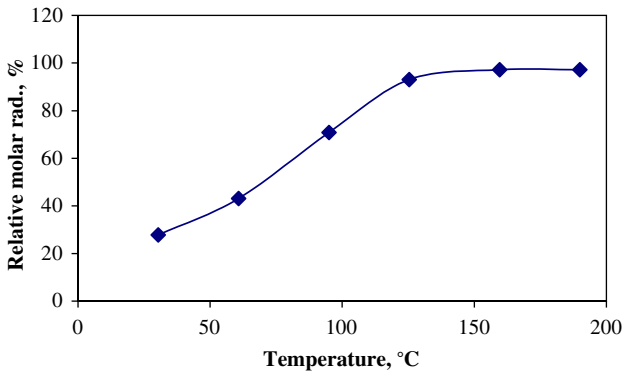

Figure 3 Relative molar radioactivity of 3-indolylacetic acid (5% $Pd/CaCO_3$–acid ratio is 3:1) at various temperatures.[13] (This figure is available in colour online at www.interscience.wiley.com/journal/jlcr.)

Introduction of a tritium label into compounds of lipid nature by solid-phase isotope exchange

Compounds of lipid nature vary appreciably in thermal stability and in stability against hydrogenolysis; they can be either polar or non-polar and either aliphatic or aromatic (Figures 8 and 9).

To optimize the conditions of labelling in compounds of this type, the degree of isotope exchange has been

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Table 5 Relative molar radioactivity of stearic acid at various temperatures, reaction time and type of the catalyst (catalyst–substrate ratio is 20:1)[14]

Time (min)	Relative molar radioactivity (%)		
	10% Pd/C (190°C)	5% Pd/C (190°C)	5% Pd/$BaSO_4$ (240°C)
1	—	21	—
2	—	—	—
3	—	30	—
4	—	—	—
5	34	45	14
7	—	49	—
8	—	—	24
9	—	63	—
10	63	78	44
11	—	78	—
13	—	76	32
15	100	57	31
17	—	—	—
20	88	—	31
25	—	—	
30	76	43	27
40	—	—	24
60	45	28	—
90	33	—	—

Table 6 Relative molar radioactivity of stearic acid at various temperatures and catalyst type (catalyst–substrate ratio is 10:1, τ= 15 min)[14,15]

T (°C)	Relative molar radioactivity (%)			
	10% Pd/C	5% Pd/C	5% Pd/$BaSO_4$	5% Rh/C
140	49	—	—	2
150	—	—	—	2
160	—	—	—	4
170	58	4	—	2
180	96	—	39	2
190	100	41	45	—
200	91	24	80	1
210	—	28	64	1
220	38	21	46	2
230	31	11	43	1
240	28	—	34	

studied as a function of the following variables: temperature (in the 60–240°C region), the catalyst:substance ratio, the reaction time (in the range of 5–60 min), the nature of the catalyst (5% Pt/C, 5% Pd/C, 5% Rh/C, 5% Pd/$BaSO_4$) and some others.

By solid-phase reactions carried out under the optimal conditions selected by the optimization technique, labelled specimens with various structures have been prepared (Tables 7–9).

Not every substance withstands the conditions of the solid-phase isotope exchange to the same extent. The

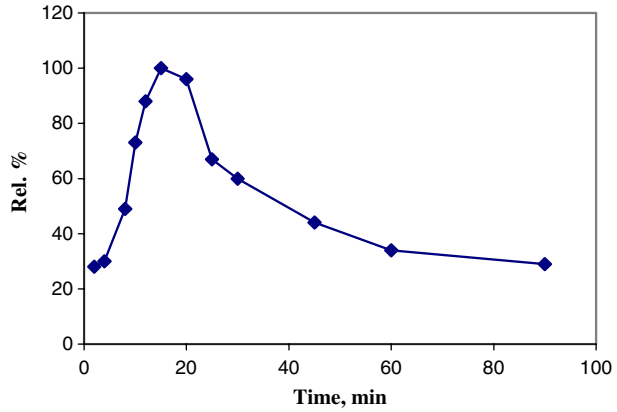

Figure 4 Degree of stearic acid labelling on reaction time (5% Pt/C, 210°C). (This figure is available in colour online at www.interscience.wiley.com/journal/jlcr.)

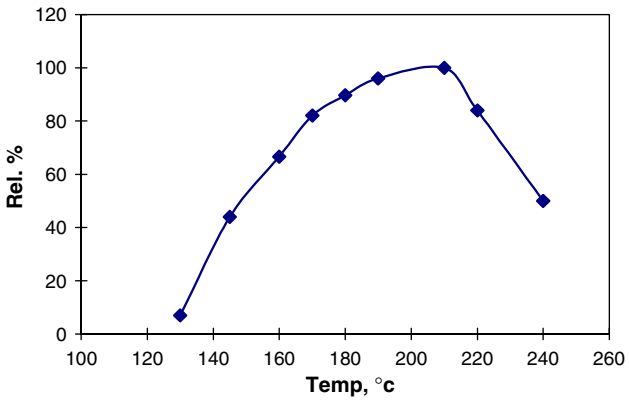

Figure 5 Degree of stearic acid labelling at various temperatures (5% Pt/C, 210°C). (This figure is available in colour online at www.interscience.wiley.com/journal/jlcr.)

chemical yield of hexadecane and cetyl alcohol after the exchange is 80%, whereas labelled compounds containing free amines, neuraminic acid and so on are formed in less than 20% yields.[20,27,32] The stabilities of the specimens can be sharply different even in the case of compounds with similar structures (Table 9). For example, the yield of the sodium salt of *N*-acetylneuraminic acid α-methyl glycoside at 200°C is almost twice as high as that of the derivative of the trisaccharide sialyllactose at 160°C.[33]

The large scatter in the yields of labelled specimens is also typical of aromatic compounds. The compounds containing triazole, pyrazole and thiazole fragments are more stable during the solid-phase method of labelling than isoxazole derivatives; however, the yields of these compounds under the conditions optimal from the standpoint of higher molar radioactivity were 2–80% (Table 8).

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The introduction of the tritium label into components of nucleic acids by solid-phase isotope exchange

The best catalyst for introducing a tritium label into purine bases is 5% $Pd/CaCO_3$. The dependence of the molar radioactivity on the reaction temperature for these compounds shows a gradual increase to 140°C and a sharp growth in the range of 150–200°C (Tables 2 and 3).[11] Apparently, the increase is due to the fact that tritium atoms start to play a noticeable role in the isotope exchange, instead of the cations.

The following labelled compounds were synthesized in the optimized conditions (Table 10).

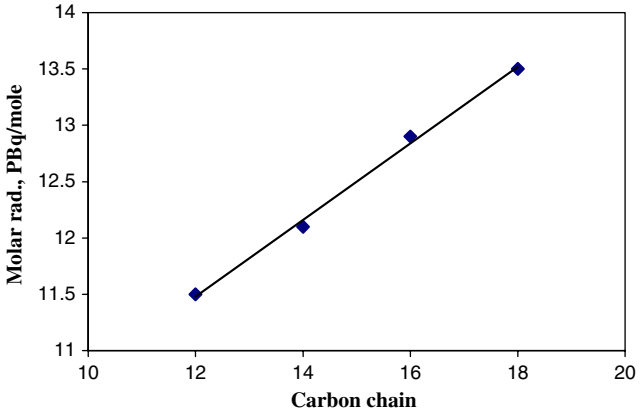

Figure 6 Dependence of the specific activity on the chain length for free fatty acids (5% Pt/C, 210°C). (This figure is available in colour online at www.interscience.wiley.com/journal/jlcr.)

High degrees of replacement of protium by tritium were attained in compounds with a rather simple structure. In the case of polymeric derivatives or compounds containing labile fragments, labelling had to be performed at relatively low temperatures, which substantially influenced the molar radioactivities of the labelled preparations. The major side products obtained according to this procedure were those resulting from hydrogenation, isomerization and destruction processes.

To increase the molar radioactivity of labile compounds, additional supports were used; this made it possible to carry out the reaction at higher temperatures (Table 11).

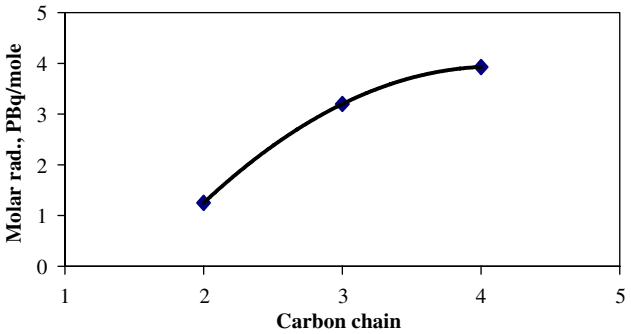

Figure 7 Dependence of the specific activity on the chain length for short-chain carboxylic acids (sodium salts). (This figure is available in colour online at www.interscience.wiley.com/journal/jlcr.)

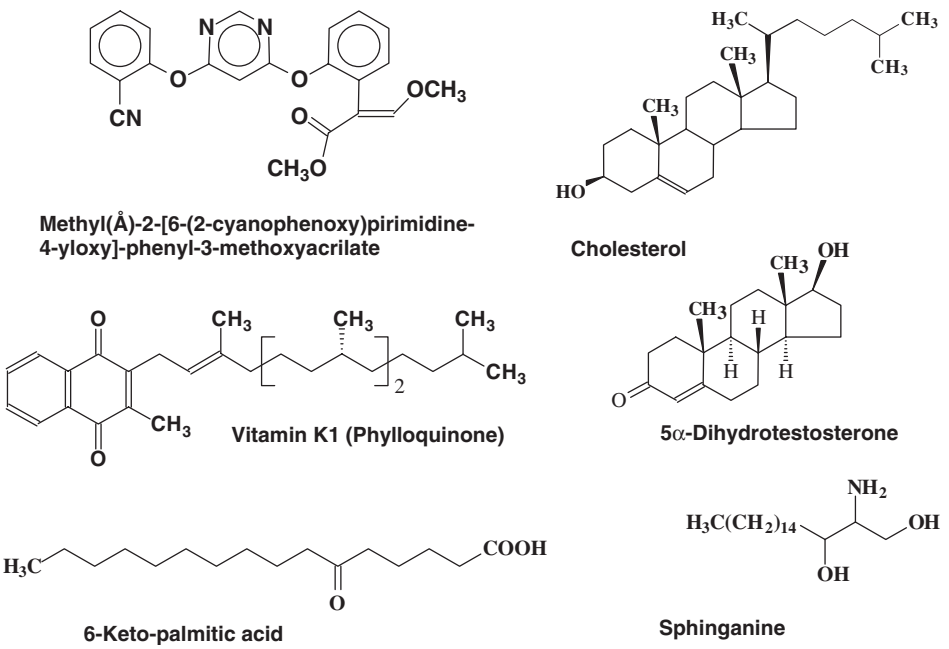

Figure 8 Structures of compounds of lipid nature.

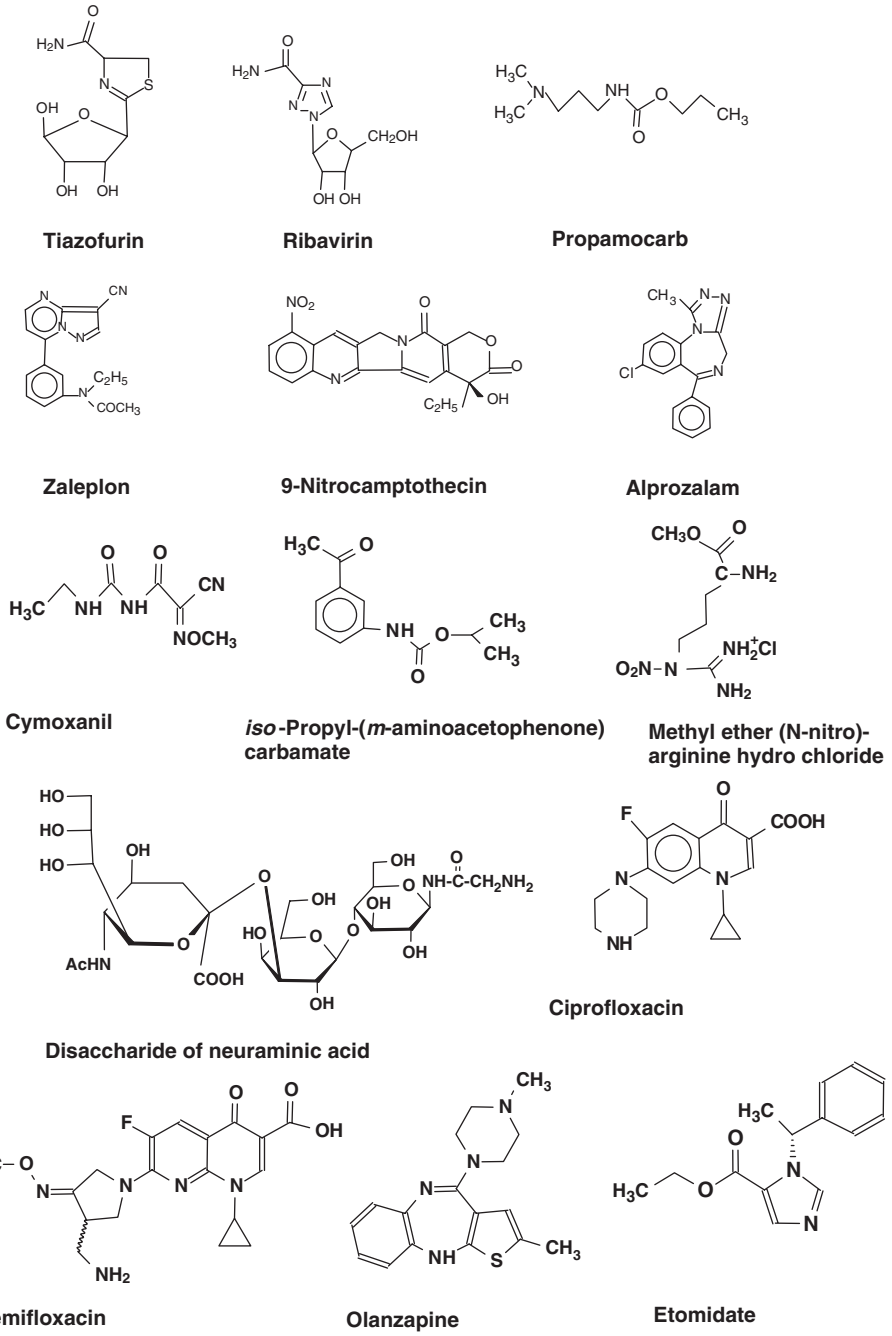

Figure 9 Structures of pharmaceuticals.

It can be seen from the data given in the table that an increase in the catalyst:compound ratio entails an increase in the degree of tritium inclusion with a simultaneous decrease in the yield of the labelled product. With the use of an additional support and a mixture of 5% $Pd/CaCO_3$. with indol-3-ylacetic acid supported on Al_2O_3, the yield of the desired product increased to 12.5%, and the specific activity of the labelled compound was as high as 75% from the theoretical value.

The introduction of the tritium label into amino acids and peptides by solid-phase isotope exchange

Many amino acids (Figure 10) are thermally more stable than nucleic acids.[35]

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At high temperatures, the degree of isotope replacement is of the same order of magnitude at any atom of the amino acid. Other data have been obtained at lower temperatures (Table 12).

Table 7 Specific activity of aliphatic compounds[14,18–29]

Compound	Sp. act. (PBq/mol[a])
6-Ketopalmitic acid	27.5–27.8
Methyl stearate	38.0–40.0
Cetyl alcohol	5.0–5.5
Hexadecane	17.0–17.5
Cholesterol	0.5–1.0
5α-Dihydrotestosterone	0.52–0.56
5α-Dihydrotestosterone enanthate	1.30–1.50
Sphinganine	7.78

[a]The specific activity of the specimen caused by the introduction of one tritium atom is 1.08 PBq/mol.

Table 8 Specific activity of aromatic compounds (palladium catalyst, $\tau = 15$ min)[15,19,22,28,30,31]

Compound	*T* (°C)	MR (PBq/mol)	Yield (%)
3-(3-Pyridyl)phenyl-isoxazole	60	0.0004	25
Alprazolam	175	0.15	4
Camptothecin	190	0.96	7
Tiazofurin	155	0.67	55
Ribavirin	160	1.11	80
Isopropyl-*N*-(m-acetyl-phenyl) carbamate	140	1.32	30
Gemifloxacin	180	0.34	5
Olanzapine	200	0.63	10
Zaleplon	180	0.19	2

The distribution of tritium in the (R)-4-hydroxy-[G-^{3}H]-L-proline molecule was studied. This compound was synthesized by heating (150°C) for 60 min a mixture of 5% Rh/Al_2O_3 with the activated Norit carbon impregnated with an aqueous solution of amino acid and then freeze-dried (the catalyst:Norit:(R)-4-hydroxy[G-^{3}H]-L-proline ratio was 10:50:1).

Hence, when the solid-phase isotope exchange is carried out under relatively mild conditions, the following tendency can be traced: the lower the activation energy for the exchange at a given carbon atom, the higher the degree of replacement of protium by tritium (Table 11).

The replacement of protium by tritium is usually higher for the methyl groups of amino acids than for the methylene groups or than the isotope exchange at a tertiary carbon atom, which also confirms a hypothesis that $^3H^+$ are the active particles in spillover.[37]

In the case of amino acids, the use of an additional support also promoted an increase in the molar radioactivity (Tables 1 and 13). Activated carbon, alumina and calcium carbonate are used most often.

The method according to which a mixture of the substance with the catalyst is treated before the reaction by transition metal salts also proved to be efficient for increasing the molar radioactivity of thermally unstable preparations (Table 1). For example, rhodium salts were used in the labelling of amino acids.[30,37] It was shown that $RhCl_3$ not only stabilizes labile compounds but also simultaneously, modifies the initiator of hydrogen spillover. Apparently, co-precipitation of rhodium salts on palladium with

Table 9 Conditions of tritium labelling of low-molecular weight bioregulators[12,30,32]

Compound	Reaction conditions			
	Catalyst	*T* (°C)	MR (PBq/mol)	Yield (%)
4-Ethyl-2,6,7-trioxa-1-phosphabicyclo-[2.2.2]octane 1-oxide	5% Pt/C	170	1.15	14
Sodium salt of *N*-acetylneuraminic acid oc-methyl-glycoside	5% $Pd/CaCO_3$	200	0.46	25
Derivative of the trisaccharide sialyllactose	5% $Pd/CaCO_3$	160	0.32	15
Propamocarb	5% $Pd/BaSO_4$	135	0.56	58
Cymoxanil	5% $Pd/CaC0_3$	90	0.006	30

Table 10 Nucleic acids synthesized by solid-phase exchange reactions with tritium gas[11,33,34]

Compound	Catalyst	Temperature (°C)	Yield (%)	Sp. act (PBq/mol)
Kinetin	5% $Pd/BaSO_4$	140	12.5	5.56
6-Benzylamino-purine	5% $Pd/BaCO_3$	190	13	5.74
Guanine	5% $Pd/CaCO_3$	210	75	0.83
Xanthine	5% $Pd/CaCO_3$	210	75	0.93
Hypoxanthine	5% $Pd/CaCO_3$	210	67	1.90
Adenosine	5% $Pd/CaCO_3$	210	93	0.89
2′-Deoxy-Guanosine	5% $Pd/CaCO_3$	210	17	1.56

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Table 11 Relative molar radioactivity and yield of labelled indol-3-ylacetic acid obtained by labelling under various conditions[13]

Reaction conditions				RMR (%)	Yield (%)
I[a]	II[b]	III[c]	T (°C)		
Catalyst: 5% *Pd/CaCO*$_3$					
5:1	—	—	150	17.1	1.9
10:1	—	—	150	40.0	0.7
20:1	—	—	150	53.3	0.4
50:1	—	—	150	66.7	0.2
5:1	Al_2O_3	10:1	200	100	12.5
Catalyst: 5% *Pd/BaSO*$_4$					
5:1	$CaCO_3$	10:1	200	84.4	3.2
5:1	$CaCO_3$	50:1	200	—	—
5:1	Al_2O_3	10:1	200	84.0	10.2
5:1	Al_2O_3	50:1	200	44.0	6.0
Catalyst: 5% *Pd/Al*$_2$*O*$_3$					
5:1	Al_2O_3	10:1	200	55.6	5.3
5:1	Al_2O_3	50:1	200	56.0	1.9

[a]The catalyst:substance ratio (mg/μmol).
[b]Additional support.
[c]The additional support:substance ratio (mg/mg).

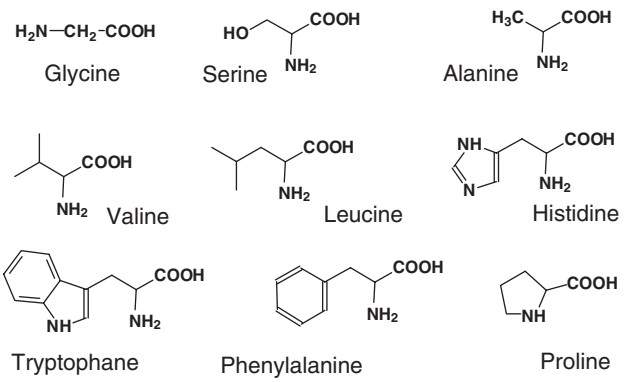

Figure 10 Structures of some amino acids.

Table 12 The ratio of the activation energy (E_a) of hydrogen exchange at a given carbon atom to the activation energy (E) of hydrogen exchange in methane is as follows[36]

Position of the label	E_a/E	Content of the label (%)
3β	0.946	1
3α	0.856	6
5α	0.653	59
5β	0.794	21
2	0.852	9
4	0.924	4

subsequent heating under an atmosphere of tritium gas gives rise to bimetallic active sites, which acquire new properties of both an adsorbent and an initiator of hydrogen spillover. Phenomena of this type have been considered in detail in a study of the influence of technetium on the efficiency of solid-phase isotope exchange.[39]

Comparison of the catalytic activity of mono- (Pd or Pt) and bimetallic systems (Pd–Tc or Pt–Tc) showed a non-additive increase in the catalytic activity (synergism) in the case of bimetallic catalysts with respect to the activity of metal catalysts used separately under the same conditions.[40] Some researchers explain the synergistic effect by the fact that hydrogen is activated on one type of active sites and the reactions proceed on another type of active sites.[40–43] Other researchers believe that the synergism is mainly related to the interaction of the atoms of one metal catalyst with the other.[44–47] In this case, hydrogen becomes activated and reacts in the same phase, i.e. the activated hydrogen species migrate from one metal atom to the other within the bimetallic active site.

Peptides are more labile compounds than amino acids. In addition, there exists the problem of racemization of amino acid residues at the α-position during the labelling. Therefore, labelled peptides are obtained by conducting reactions at relatively low temperatures (Table 14).

The distribution of the label in the amino acid residues in these peptides was approximately equal, but hydrogen at the α-carbon atoms of amino acids proved to be the most reactive in the isotope exchange with tritium. For example, the peptide Met-Gly-His-Phe-Pro-Gly-Pro contained after labelling (140°C), on average, 1.44 tritium atoms, and 88% of them were in the α-positions of amino acid residues. At 180°C, 4.29 tritium atoms were incorporated in this peptide, and only 76% of tritium occurred in the α-position of the amino acid residues.[49] Thus, the same trend is observed: the higher the reaction temperature, the more uniform the distribution of the label (Table 15).

The influence of the peptide structure on the distribution of the label among amino acid residues was studied using model tripeptides (Table 16).

It can be seen from the data listed in Table 15 that at 150°C uniformly. For example, the amounts of tritium in Gly(I) and Gly(II) in the Gly-Gly-Val peptide differ by a factor of 5. When tritium is introduced in identical amino acid residues, the label is mainly found in terminal amino acids; in the case where both residues are terminal, the label is inserted predominantly into the amino acid residue located at the *N*-end of the peptide. The patterns of label distribution in a free amino acid molecule and in the same molecule incorporated in a peptide are similar.

During labelling of more complex amino acid sequences, it may happen that one fragment of the

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Table 13 Molar radioactivity of biotin and amino acids prepared by solid-phase isotope exchange using various additional supports[10]

Compound	Catalyst	The catalyst:substance ratio (mg/mg)	Additional support (mg/mg)	T (°C)	MR (PBq/mol)
Biotin	5% $Pd/CaCO_3$	10:1	Al_2O_3, 10:1	200	1.6
L-Phenylalanine	5% $Pd/CaCO_3$	16:1	Al_2O_3, 5:1	200	3.03
L-Methionine	5% Rh/Al_2O_3	10:1	Al_2O_3, 10:1	200	3.30
L-Histidine	5% $Pd/BaSO_4$	10:1	$CaCO_3$, 10:1	220	4.60

Table 14 Molar radioactivity of tritium-labelled peptides (T= 140°C, labelling was carried out using an additional support or the rhodium complex with the peptide)[49]

Peptide	MR (PBq/mol)	Peptide	MR (PBq/mol)
Tyr-D-Ala-Gly-Phe-Leu-Arg	1.68	Gly-Asn-NH_2	1.42
Thr-Lys-Pro-Arg-Pro-Gly-Pro	1.96	Tyr-Ala-Gly-Phe-Leu	1.67
Met-Gly-His-Phe-Pro-Gly-Pro	1.74	Tyr-Gly-Gly-Phe-Leu-Arg	1.11
Tyr-Ala-Gly-Phe-Tyr-Pro	1.63	Tyr-Gly-Gly-Phe-Leu	0.89
Gly-Leu-Leu-Asn-Leu-Lys	1.40	Met-Glu-His-Phe-Pro-Gly-Pro	1.69
Tyr-Pro-Arg	1.44	Tyr-Pro-Pro-Gly-Gly-Ser-Lys-Val-Ile-Leu-Phe	2.33
Tyr-D-Ala-Gly-Phe-Gly(OH)	1.78		

Table 15 Degree of labelling of His-Gly(I)-Gly(II) at various temperatures[37]

Amino acid	Temperature (MR/PBq/mol)					
	135 (1.85)		150 (4.07)		170 (6.30)	
	α	β	α	β	α	β
His	86	17.2	72	14.4	63	12.6
Gly(I)	5	2.5	12	6	16	8
Gly(II)	9	4.5	16	8	21	10.5

α, the fraction of the label in the given amino acid (%);β, the fraction of the label in the same amino acid per hydrogen atom (%).

Table 16 Distribution of tritium in labelled tripeptides (T= 150°C)[44]

Peptide	MR (PBq/mol)	Yield (%)	Label distribution (%)		
			His or Val	Gly(I)	Gly(II)
His-Gly-Gly	4.07	40	72	12	16
Gly-His-Gly	2.41	25	62	30	8
Gly-Gly-His	2.59	12	57	29	14
Val-Gly-Gly	2.59	45	72	10	18
Gly-Val-Gly	1.41	30	13	59	28
Gly-Gly-Val	1.74	32	6	79	15

molecule shields another fragment from the reaction with active tritium species. In this case, the distribution of the label along the amino acid chain is no longer uniform.[50]

For example, the distribution of the label in α-conotoxin Gl (Table 17).

The Asn^4-Pro^5-Ala^6 fragment contained less than 2% of the label. Apparently, the low reactivity of these residues is related to the spatial structure of this peptide.

Approaches to introducing a label into proteins have been developed (Table 18).

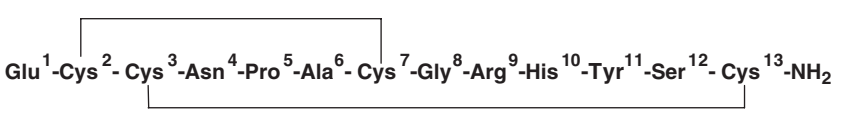

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Table 17 Distribution of the label in α-conotoxin G1 (5% Rh/Al_2O_3–peptide–Al_2O_3 ratio was 5:2:50, T= 140°C, t= 20 min, the molar radioactivity of the labelled preparation – 1.30 PBq/mol)

Fragment of amino acid	Content of the tritium label (%)	Fragment of amino acid	Content of the tritium label (%)
Glu1	3.33	Arg9	1.64
Cys2	6.80	His10	50.56
Cys3	8.93	Tyr11	4.08
Cys7	7.80	Ser12	3.55
Gly8	2.61	Cys13	8.92

Table 18 Preparation conditions and radiochemical and biological characteristics of tritium-labelled proteins[37]

Proteins	T (°C)	Yield (%)	Specific radioactivity (mCi/mg)	Biological activity (%)
Collagenase	80	25	25	70
Collagen	60	10	8	—
Lichenase	60	40	28	80
β-Glucosidase	20	55	7	86
	40	40	20	75
	60	30	70	28
	80	10	200	12

Table 19 Distribution of the label in benzoic and salicylic acids introduced by the Wilzbach method (upper) and by treatment with tritium gas of the acids applied on the heterogeneous catalyst (lower)[51]

Acid	Content of the label (%) in the position		
	Ortho	Meta	Para
Benzoic	58.3	21.3	20.4
	5.0	72.5	22.5
Salicylic	39.0[a]	48.4[a]	13.6[a]
	16.9[a]	31.7[a]	51.4[a]

Note: The value indicated above the bar was obtained using method I and that below the bar was obtained using method II. [a] Relative to the hydroxy group.

Table 20 Distribution of the label in the aromatic rings of tyrosine and serotonin (relative to the hydroxy group, at 150°C) [27]

Compound	The contents (%) of the label in the aromatic rings (the total amount of the label in the ring was taken to be 100%) were as follows	
	Ortho-position	Meta-position
Tyrosine	78	22
Serotonin	85	15

Experimental data obtained for solid-phase isotope exchange reactions of organic compounds allow to conclude that $^3H^+$ ions can be considered as a basic type of active particles.

This can be illustrated using the distribution of the label in aromatic rings of benzoic and salicylic acids, tyrosine and serotonin as examples (Tables 19 and 20).

It can be seen from these data that electrophilic substitution predominates in the replacement of hydrogen in the aromatic ring by tritium using heterogeneous catalysts, whereas in labelling by the Wilzbach method, radical substitution is the prevailing process. It is also significant[52] that upon solid-phase labelling of a peptide with *N*-terminal tyrosine at a rather low temperature (135°C), the amount of the label inserted in the ortho-position relative to the hydroxy group of tyrosine was ~20 times as high as that in the meta-position.

These data provide grounds for two conclusions. At first, the molar radioactivity of labelled specimens at relatively low temperatures (usually below 180°C) is mainly determined by reactions of the organic compound with tritium cations. At second, the lower the reaction temperature, the higher the predominance of electrophilic substitution, but at higher temperatures, the contribution of atomic tritium in the labelling increases.

The possibility of introducing a label into organic substances susceptible to hydrogenolysis is also largely determined by the mechanism of this process. It is known that liquid-phase reactions proceed in the active sites of the catalyst. These sites adsorb, first of all, unsaturated fragments of molecules; hence, hydrogenolysis and hydrogenation are the predominant types of reactions. Under solid-phase conditions, labelling is due to reactions of molecules of the substance with activated tritium. Apparently, reduction of the N–CHR–C_6H_5 and O–CHR–C_6H_5 fragments to toluene or its derivatives or reduction of the NO_2, = N–OR groups to amino groups, etc. would be hampered under solid-phase reaction conditions, as nitrogen and oxygen atoms are prone to be protonated and isotope exchange can be carried out selectively without disturbing the native structures of biologically active products (Table 21). An important feature of solid-phase hydrogenolysis is the retention of the initial structure of compounds including chirality (Table 22).

Preserving of the chirality in the hydrogen isotope exchange solid-phase reactions can be explained by intramolecular mechanisms of electrophilic aliphatic substitution.[55–57]

Solid-phase hydrogenation with tritium

Heterogeneous catalytic hydrogenation is yet another method for preparing tritium-labelled compounds. Solid-phase hydrogenation is carried out by the same

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procedures as the solid-phase isotope exchange. The solid-phase hydrogenation reactions possess a number of features inherent in the isotope exchange reactions, but they also have some distinctive features. For example, hydrogenation of unsaturated carbon–carbon bonds proceeds even at room temperature, while unsaturated carbon–eteroatom bonds are usually hydrogenated under more rigorous conditions (Table 23).

If investigations require labelled preparations with molar radioactivities much higher than those normally obtained by hydrogenation of double bonds, the solid-phase hydrogenation procedure is more efficient than the standard liquid-phase procedure. This is due to the fact that hydrogenation carried out with heating is accompanied by isotope exchange.

For the solid-phase hydrogenation of unsaturated carbon–heteroatom bonds, thorough selection of the labelling conditions is required. For example, the nitrile of glycolic acid was used as the precursor in the synthesis of labelled ethanolamine. This precursor was reduced in the presence of 5% Pt/C, 5% Pd/C, 5% Rh/Al_2O_3 and 5% $Rh/CaCO_3$.[58,59] The 5% Rh/C catalyst (Tables 24 and 25), a temperature of 100°C and a reaction time of 20 min were found to be the best conditions as regards the synthesis of a highly labelled specimen (1.04–1.48 PBq/mol).

Table 21 Molar radioactivities of labile compounds labelled in the presence of heterogeneous platinum group metal catalysts. [53,54]

Compound	*T* (°C)	MR (PBq/mol)
9-Nitrocamptothecin	190	0.59
Dimethomorpholine	160	0.24
Thyroxin	180	0.25
Methyl ester of *N*-nitroarginine	180	0.85
Etomidate	160	0.86
Vitamin K_1	130	2.3

Table 22 Molar radioactivity, yield and optical purity of labelled amino acids prepared by solid-phase isotope exchange (200°C, 60 min)[55]

Compound	MR (PBq/mol)	Yield (%)	Optical purity (%)
Glycine	1.8	90	—
Serine	1.4	50	75
Alanine	3.2	65	82
Valine	6.5	85	94
Leucine	7.9	70	85
Isoleucine	8.1	75	88
Histidine	4.5	20	50
Tryptophane	3.6	30	70

Table 24 Relative molar radioactivities of the specimen prepared by the reduction of glycolic acid nitrile (catalyst 5% Rh/C, the catalyst:substance ratio is 5:1, reaction time is −20 min) at different temperatures

T (°C)	RMR (%)	*T* (°C)	RMR (%)
20	11	120	85
50	31	140	66
70	55	160	33
90	67	180	13
100	100	190	6

Table 25 Relative molar radioactivities of the specimen prepared by the reduction of glycolic acid nitrile (catalyst 5% Rh/C, the catalyst:substance ratio is 5:1, reaction temperature is −100°C) at different reaction time

τ (min)	RMR (%)	τ (min)	RMR (%)
5	26	50	57
10	50	60	29
15	80	75	16
20	100	90	11
30	92	120	9
40	78	150	6

Table 23 Molar radioactivity and yields of labelled compounds obtained by tritium labelling using solid-phase hydrogenation[15,19,20,26]

Starting compound	Reaction conditions			MR[a]	Yield (%)[b]
	Catalyst	*t* (°C)	*T* (min)		
Allyl alcohol	5% $Pd/BaSO_4$	20	30	2.20	76
But-3-en-1-ol	5% $Pd/BaSO_4$	20	30	1.95	85
11-Cyanoundecanoic acid	5% Rh/C	80	180	2.05	51
2,2,6,6-Tetramethyl-4-oxopiperidine	5% Rh/C	80	180	4.80	57
2,2,6,6-Tetramethyl-4-hydroxyiminopiperidine	5% Rh/C	100	180	4.80	20
Benzylamine	5% Rh/Al_2O_3	60	180	7.78	45
(lR,2R)-l-Amino-2-(4-methylpiperidinylmethyl)-cyclohex-4-ene	5% Pd/C	120	15	1.15	2
Methacrylic acid	5% Pd/C	170	20	5.63	80

[a] Expressed in PBq/mol.
[b] The yield of a reduced or hydrogenated product.

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In addition, by using solid-phase hydrogenation, one can increase the yield of the labelled specimen (with respect to the liquid-phase method). This situation is often observed when hydrogenation affords isomeric products as, for example, in the reduction of 16α,17α-cyclo-hexanopregn-4-ene-3,20-dione. The reaction gives rise to saturated steroids, namely, the 5α and 5β-16α,17α-cyclohexanopregnane-3,20-dione isomers, in a ratio of 1:14 by using liquid-phase method and a ratio of 1:3 by using solid-phase hydrogenation.[60]

Solid-phase dehalogenation

Catalytic dehalogenation is the best-known hydrogenolysis reaction. If tritium gas is used as the reagent, dehalogenation gives rise to tritium-containing compounds.

Usually, aromatic compounds are dehalogenated by a liquid-phase procedure.[60] This affords labelled specimens with a molar radioactivity of 0.7–0.9 PBq/mol. However, the liquid-phase approach cannot be used, as a rule, for labelling of saturated halogen-containing compounds under conditions acceptable for work with tritium gas. The advantages of the solid-phase approach have been demonstrated in relation to the dehalogenation of aliphatic compounds.[19]

The solid-phase catalytic dehalogenation was first employed to introduce a tritium label into uracil.[1] Characteristic features of this reaction as applied to tritium labelling of nucleic acid components have been studied in detail (Figure 11).

It has been shown that the degree of dehalogenation increases with an increase in the palladium content on carbon or with an increase in the catalyst to substance ratio.

The selectivity problems in the solid-phase hydrogenolysis by tritium

This section considers approaches designed for solid-phase labelling either by isotope exchange when the substrate molecules contain readily reducible fragments (for example, nitro groups or double bonds) or by selective dehalogenation and hydrogenation (Table 21). The selective hydrogenation can involve either hydrogenation of one or several double bonds or hydrogenation of one aromatic fragment in the presence of several other such fragments.

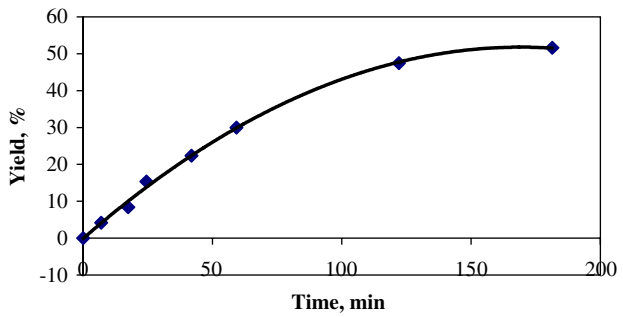

Figure 11 Kinetics of dehalogenation of 8-bromoguanosine at 56°C.[33,60] (This figure is available in colour online at www.interscience.wiley.com/journal/jlcr.)

For selecting the optimal conditions for labelling compounds with double carbon–carbon bonds, a special study has been carried out to establish the temperatures of reduction of double bonds with different degrees of substitution.[53]

Fusicoccin was chosen as the model compound to elucidate the conditions of labelling by selective solid-phase hydrogenation of one double bond.[61–63] because this compound contains mono-, tri- and tetra-substituted double bonds. Selective solid-phase hydrogenation was studied in the temperature range of 40–220°C, the reaction time and the pressure of tritium gas being varied (Table 26).

It can be seen from the above data that the selective hydrogenation of a monosubstituted double bond starts even at 40°C and proceeds efficiently at 100°C. An increase in the pressure of tritium gas and the reaction time affect to a lesser extent the process selectivity (Table 26).

The product ratio (fusicoccin–DHF–THF) in the reaction mixture was changed from 19:26:1 to 0.1:3.4:1 when the temperature raised from 100 to 120°C. Increasing the reaction time by a factor of 3 changes the ratio to 1.5:17.2:1; and changing the tritium pressure from 210 to 350 hPa to 4.3:20:1.

Accordingly, unsaturated compounds with three- and tetra-substituted double bonds could survive in solid-phase reactions with gaseous tritium until temperatures about 100°C.

Thyroxin was used as the halogen-containing compound to study the possibility of labelling by selective dehalogenation. At high temperatures (180–200°C), liothyronine and iso-liothyronine could be isolated from the reaction mixture in a pure state. At 180°C (τ= 10 min), the yields of these compounds were

Table 26 Dependence of the content of major hydrogenation products of fusicoccin in the reaction mixture on the pressure of tritium gas and the reaction time (T= 100°C, the Lindlar catalyst:substance ratio is 10:1)[53,64]

Reaction conditions, pressure (hPa) (Tmin)	Fusicoccin	[$^3H_2$]DHF	[$^3H_4$]THF
210 (20)	38	51	2
280 (20)	32	57	2
350 (20)	13	60	3
350 (60)	6	69	4

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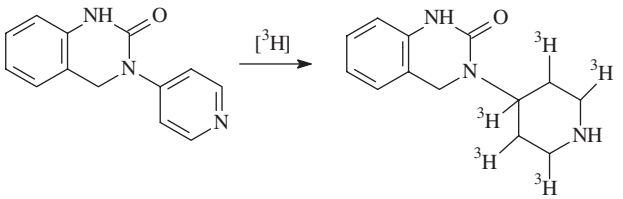
3.2 and 0.4%, and at 200°C (τ= 5 min), they were 5.3 and 1.3%, respectively. The molar radioactivity of the dehalogenated products reached 0.7 PBq/mol.[53]

Optimization of solid-phase hydrogenation and dehalogenation is usually the necessary step for studying the stability of a particular labile fragment in the molecule as, for example, in the case of vitamin K_1 with a trisubstituted double bond in the molecule.[53]

An absolutely different situation occurs in working out the conditions of selective hydrogenation of one aromatic fragment in the presence of other aromatic fragments (for example, the pyridine and benzene rings). Hydrogenation of aromatic compounds in solution is known to occur only at very low pH.[26,65] However, this results in high isotope dilution of the labelled specimen. For example, in the synthesis of cyclohexylmethylamine from benzylamine using the liquid-phase hydrogenation procedure, the molar radioactivity of the labelled preparation was 1.66 PBq/mol, whereas the solid-phase hydrogenation procedure gave a specimen with a molar radioactivity of 7.78 PBq/mol. In this case, the advantage of the solid-phase approach is obvious. Apparently, the nature of the activated tritium species is largely responsible for whether or not the process can, in principle, be selective. If these species are cations, the process selectivity would be high (the pyridine fragment is protonated much more efficiently than the benzene fragment). The aromatic structure of the pyridine fragment is violated and hydrogenation is thus facilitated. For example, in the case of selective hydrogenation BIBN 4447 the labelled molecule contained five tritium atoms (6.44 PBq/mol) (Scheme 1).[12]



Scheme 1

The content of this product in the reaction mixture was 7 times as high as the total content of all other substances, i.e. the process was highly selective. This indicates that tritium cations play an active role in the hydrogen spillover in the given conditions.

Preparation of tritium-containing compounds on the basis of reagents labelled by the solid-phase method

In some cases, it proves to be more difficult to introduce a label into a biologically active compound than into one of its fragments. In these cases, the label is introduced into this fragment and then the structure of the molecule is reconstituted. For example, *N*-oleoyldihydrosphingosine with a high molar radioactivity was synthesized in two stages. First, dihydrosphingosine was labelled by a solid-phase procedure,[27] then the product was condensed with oleic acid by the method of mixed anhydrides (Scheme 2).

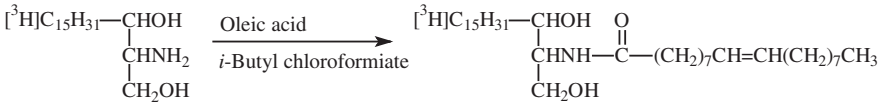
2-Hydroxyethylamides of eicosapolyenoic acids were synthesized in a similar way. Evidently, it is impossible to introduce any significant amounts of tritium into the final compound without affecting the double bonds of polyunsaturated fatty acids. Therefore, solid-phase tritiation of glycolic acid nitrile was used to obtain aminoethanol,[57] which was then condensed with a fatty acid by the method of mixed anhydrides (Scheme 3).

The unlabelled reagent was taken in a tenfold excess.

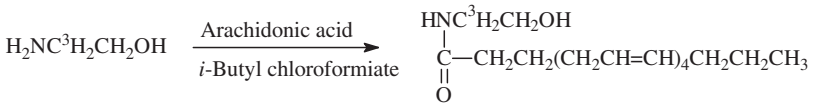
Discussion

It can be seen from the given data that the mechanism of reactions between tritium gas and an organic substance applied on a heterogeneous catalyst considerably differs from that involved in labelling by the Wilzbach method and its numerous modifications such as activation with γ-rays, Tesla discharge, UV light, etc.

Usually, this is related to the processes taking place when catalysts are saturated with hydrogen.[66–68] According to a view proposed and substantiated on the basis of a study of the valence state of hydrogen in



Scheme 2



Scheme 3

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$$H_2 + MM \rightarrow H^{\delta+} + MM^{\delta-} \rightarrow H^{\delta\cdot-} + MM^{\delta\cdot+}$$
(where MM is metallic catalyst particle)

intermetallic hydrides,[66] hydride dispersion can be considered as being due to a redox process giving rise to hydride ions $H^{\delta-}$, which takes place when the hydrogen concentration (C_H) in the solid hydride is higher than a critical concentration C_{Hcr}. The C_{Hcr} value is determined by the redox potential of the reaction where MM is the metallic matrix of the catalyst.

According to this model, hydrogen chemisorbed on the active sites of the catalyst surface donates some of its s-electrons to the conduction band of the metal. Therefore, at low C_H values, the behaviour of absorbed hydrogen is described satisfactorily by the Ubbelode model,[67] which implies high diffusional mobility of $H^{\delta+}$ and an increase (or, at least no decrease) in the electrical conductivity of the hydride phase with respect to that of the initial metal. The $\tilde{N}_{Hcr}$ value corresponds to a concentration of the oxidant $H^{\delta+}$ at which these species oxidize the metallic matrix and become electron density acceptors, being converted into bulky and low-mobility $H^{\delta-}$ ions, and thus deplete the conduction band of the metal. It is evident that at higher degrees of filling of voids by $H^{\delta-}$ ions having a relatively great effective volume, the electron deficiency on the metal atoms that form the metal sublattice in the crystal structure of the catalyst should increase, which may restrict the reversible adsorption of hydrogen. This sharply decreases the concentration of activated species of tritium able to enter into exchange reactions and hence arrests almost completely the isotope exchange.[54] The C_{Hcr} value may not be attained for a long period when hydrogen spillover takes place.

The transfer of activated species from the metal crystallite to the support has been the subject of numerous publications[69–71] which evaluate how this process is affected by adsorption of molecular hydrogen, extensive rearrangement of the sorbent surface, relaxation of disordered structures and other factors. The migration of a proton over the oxygen-containing surface of the support follows a relay mechanism. The activation energy of this transfer equals 20.1 kJ/mol.[72]

Study of spillover over the support surface revealed both electronic and ionic conduction.[74] Therefore, the observed process, unlike those involved in the Wilzbach method, can be described more reliably. in terms of a model we called as 'capacitor model' described in References [64,65].

According to this model, the $^3H^+$ ions and the electrons formed in the reaction of tritium gas with the catalyst migrate separately over the surface. The support surface acts as a sort of multilayer capacitor. Depending on the experimental conditions, interaction of tritium cations with electrons (discharge of the neighbouring oppositely charged zones) takes place with one or another probability, giving rise to tritium atoms, which rapidly recombine and pass to the gas phase. When the flux of $^3H^+$ ions and electrons reaches the substrate, isotope exchange, hydrogenation, dehalogenation and other reactions occur efficiently. If the substrate contains easily protonated groups, the degree of labelling should markedly diminish. It is evident that at higher temperatures, the probability of interaction between neighbouring oppositely charged zones would increase; therefore, the contribution of the reactions of atomic tritium to the processes resulting in the formation of the labelled compound would also increase.

Thus, if the substrate contains readily protonated groups, the molar radioactivity of the labelled product should markedly increase with temperature rise (the neutral species of atomic tritium would react more efficiently with these compounds).

For example, sulphobromophthalein contains easily protonated sulpho and phenolic groups, which may give rise to a substantial positive charge. Consequently, the possibility of isotopic exchange is expected to drop considerably (thus, the molar radioactivity of the labelled sulphobromophthalein did not exceed 0.08 PBq/mol at 210°C). Temperature rising up to 285°C increases the molar radioactivity to 0.5–0.6 PBq/mol.

Conclusion

This paper covers the results of synthesis of labelled specimens using solid-phase hydrogenolysis reactions including hydrogenation, dehalogenation, selective hydrogenation, chemical synthesis and isotope exchange with tritium gas. The regularities of these reactions were studied, in particular, the effects of the nature of the substrate and the catalyst, the pressure of tritium gas, the process duration, temperature, etc. on the yield and molar radioactivity of the resulting specimens. Examples of preparation of labelled biologically active compounds are cited.

It was found that at temperatures below 180°C used most often for solid-phase labelling, the key radiochemical parameters of labelled specimens (yield, molar radioactivity, tritium distribution) are governed by the reaction of $^3H^+$ with the substrate. At higher temperatures, the concentration of atomic tritium increases. In this case, especially for substrates containing easily protonated groups, the molar radioactivity of the product should markedly increase (non-charged species

DOI: 10.1002.jlcr

of atomic tritium react more efficiently with these compounds), and the distribution of the label becomes more uniform.

The service life of the catalyst depends on the time it takes to reach a certain threshold concentration of hydrogen in the crystallites of the metal catalyst, C_{Hcr}. The time required to reach this value (C_{Hcr}) is dictated by the relationship between the rate of saturation of metal crystallites with tritium gas and the efficiency of its spillover.

If the goal is to prepare a labelled specimen in a high yield and with the distribution of the label corresponding most closely to that obtained upon electrophilic substitution, the solid-phase reaction should be carried out at the lowest temperature that would ensure the required molar radioactivity. The reaction time is determined by the period of catalyst deactivation. If the aim is to obtain the labelled specimen with the highest possible molar radioactivity, the reaction is carried out in the temperature range in which the required [^{3}H]-labelled compound can be isolated in a yield of at least 5–10%. The reaction time is usually 5–15 min.

Concerning the problem of the effective synthesis of tritium labelled biologically active compounds, a rather unexpected conclusion can be drawn. Although a flow of activated $^3H^+$ particles is sufficient even at room temperature, a kinetics of electrophilic hydrogen exchange of C–H bond becomes reasonable only at temperatures 150–180°C. Hence, an extremely active catalyst usually causes side reactions and complete decomposition of the substance that makes labelling impossible. Thus, additional approaches are used to stabilize substance to activated tritium particles. As a rule, these methods decrease an activity of the catalyst, allowing the substrate to survive at temperatures to be effective for exchange reactions.

A promising expedient is to combine various catalysts (palladium–rhodium or palladium–technetium mixed catalyst), supports (separate application of the catalyst and the substrate followed by mechanical mixing) or substrates (consecutive application of several substrates). These approaches increase the yield of the labelled specimen and in some cases, the synthesis of such substances would be otherwise impossible.

It is shown experimentally that if the substrate includes easily protonated groups, label incorporation considerably drops, especially at positions neighbouring the protonate group. In addition, protonated groups become more labile (for desamination, polymerization, hydrogenation of pyridine ring, etc.), but adjacent groups can become even more stable that makes it possible to use selective solid-phase hydrogenolysis reactions for tritium labelling.

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