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Methods for the synthesis of tritium-labelled fatty acids and their derivatives, oxylipins and steroids

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Abstract. The achievements in the field of synthesis and application of tritium-labelled oxylipins, steroids, fatty acids, phospho-, sphingo- and other lipids are reviewed. The importance of these studies for the solution of current problems of biochemistry, biology and pharmacology is exemplified in the application of labelled compounds. The bibliography includes 148 references.

I. Introduction

Methods used for the synthesis of tritium-labelled classical representatives of lipids and oxylipins (fatty acids, steroids, prostaglandins, sphingo- and phospholipids, *etc*.) and their biologically active analogues close in physicochemical properties to lipids have gained considerable popularity. The choice of lipids for investigation is based on the fact that apart from the ability of these compounds to accumulate metabolic fuel, which for many decades was considered to be their sole function,[1] two other important functions have been recently assigned to them. First, they are the principal structural components of cell membranes and, second, they are bioeffectors, which control intracellular biochemical reactions, intercellular interactions and other physiological processes occurring in the organism. Evidently, the interaction of bioeffectors with their targets and, consequently, the specificity of their action are largely determined by their structure.[1]

The advantages of tritium as a radioactive label, *viz*., low radiotoxicity, long half-life (12.26 years), high molar radioactivity (1.08 PBq g-atom^{-1}), *etc*., are universally known.[2–5] The methods used for the synthesis of tritium-labelled compounds include different versions of reactions of isotope exchange, chemical and enzymic methods. The mechanisms of labelling by isotope exchange † have been considered.[5–15]

The labelling by a liquid-phase method is accompanied by reactions of isotope exchange; migration, isomerisation and hydrogenation of double bonds; selective hydrogenation of one of several double or triple bonds; dehalogenation and reduction of the corresponding functional groups. Only a careful choice of reaction conditions can result in satisfactory yields of labelled target products. An important role is ascribed to the choice of a catalyst and a solvent and determination of optimum experimental parameters (the pressure of gaseous tritium, the reaction time, the catalyst : substrate : modifying additives ratio, *etc*.).

Classical methods have limited application, since only milligram and submilligram quantities of precursors are normally used in the synthesis of tritium-labelled compounds. As a result, the catalyst : precursor ratio should markedly exceed that prescribed for selective hydrogenation using classical procedures on purely technological grounds. In contrast, the proportional increase in catalyst-modifying additives (quinoline, pyridine, *etc*.) significantly decreases the yield of the labelled target product and considerably increases the total radioactivity of the reaction mass due to incorporation of the label into side products and modifying additives, which complicates the isolation and purification of the target compound.

If the components used in biological studies are sufficiently stable at elevated temperatures, a solid-phase method where the substance applied onto the catalyst is heated in the atmosphere of gaseous tritium is the most promising approach.[9, 12, 13]

The method of isotope exchange with tritiated water in inert solvents is used for labelling compounds that are labile in hydrogenation conditions; the use of inert solvents prevents self-radiolysis of highly enriched tritiated water.[2] The latter is usually obtained by reduction of PdO with gaseous tritium. Palladium black and palladium catalysts immobilised on inert supports initiate the isotope exchange. Obviously, the more active the catalyst the higher the degree of isotope exchange with tritiated water, but the probability of degradation of the labile compound increases also.

As a rule, chemical syntheses of tritium-labelled lipids are preceded by the synthesis of the corresponding precursors [4, 5, 16–22] containing triple or additional double bonds, halogen-substituted fragments, *etc*. Treatment of these compounds with gaseous tritium in the presence of a catalyst results in the formation of a labelled product. In some cases, tritium-labelled

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† Modern methods of isotope exchange can be conventionally divided into three main group, *viz*., heterogenous catalytic isotope exchange of gaseous tritium with an organic compound dissolved in a solvent (liquid-phase methods), heterogenous catalytic isotope exchange of gaseous tritium with a solid organic compound at elevated temperatures (solid-phase methods) and heterogenous catalytic isotope exchange of an organic compound dissolved in an aprotic solvent with tritiated water.

lipids are obtained by a multistep synthesis which utilises a labelled preparation as a starting compound. This approach requires the use of methods which not only ensure high yields of target products but also make the process technologically safe.

II. Synthesis of tritium-labelled fatty acids and their derivatives

1. Fatty acids

Fatty acids differ in the structure of the carbon backbone and the number of double bonds as well as in the presence and number of other functional groups. They are present in natural subjects, predominantly as esters. Fatty acids are constituents of phospholipids, sphingolipids, glycolipids, gangliosides, glycerides, steroid esters and vitamins. It had long been thought that the sole function of fatty acids was to provide membrane integrity and the optimum level of unsaturation of tissue lipids;[23] however, it has recently been established that these compounds also regulate the enzyme activity, modulate binding of steroid hormones to their specific receptors and control gene transcription.[24–26] Indeed, a change in the fatty acid composition of membrane lipids changes the activity of membrane-bound enzymes and expression of receptors.[27]

Isotopically labelled fatty acids can be synthesised by isotope exchange, selective hydrogenation of the corresponding acetylenic precursors with gaseous tritium as well as by chemical, microbiological and other methods.[5]

The liquid-phase method of isotope exchange is the simplest procedure for obtaining unsaturated fatty acids, since it allows direct labelling of the target compound. Besides, this procedure is devoid of many drawbacks of microbiological methods, *e.g.*, difficulties in isolation of labelled preparations from homogenates, isotopic dilution of the labelled target product with endogenous products derived from microbial cultures and the loss of the label in the course of biological conversions.[4,5] For example, the molar radioactivity of labelled (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosapentaenoic (thymnodonic) acid synthesised from [8-^{3}H]stearic acid [5] using a culture of *Saprolegnia parasitica* (ATC 22284) decreased 250–400-fold in comparison with that of the original fatty acid.

The lability of alkenes in liquid-phase isotope exchange is the main property which restricts the application of this method for introducing the label into unsaturated fatty acids. It is known [3–5] that hydrogenation, *cis–trans*-isomerisation and double bond migration occur extensively even with the Lindlar catalyst. The isomerisation reactions are equally probable, while the double bond migrates by 85%–88% towards the carboxy group. However, these restrictions did not prevent the labelling of both monoenoic and polyenoic acids by varying the reaction conditions (Table 1). Some data on the distribution of the label in molecules of these compounds will be given below.

Despite the relative simplicity of the liquid-phase isotope exchange, it is of limited use, since the molar radioactivity of target products seldom exceeds 0.1 PBq mol^{-1}. Molar radioactivities of polyenoic fatty acids are much higher, if chemical methods are used. Acetylenic precursors can be prepared either from the corresponding fatty acids or by total chemical synthesis. The former method was used in the synthesis of 5-acetylenic analogues of methyl arachidonate, methyl thymnodonate, the 14-acetylenic analogue of methyl arachidonate and the 4-acetylenic analogue of methyl (4*Z*,7*Z*,10*Z*,13*Z*,26*Z*,19*Z*)docosahexaenoate (methyl docosahexaenoate). This type of synthesis is illustrated in the example of 5-acetylenic analogues of eicosapolyenoic acids and the 14-acetylenic analogue of methyl arachinodate (Schemes 1 and 2).[16,17,30]

The major difference in these schemes is that in the latter the epoxy acid is formed upon treatment of the starting compound with *m*-chloroperbenzoic acid in dichloromethane in the presence of $KHCO_3$.[16] It is known that the use of nonpolar solvents for epoxidation results in the formation of substantial amounts of diepoxides due to the high reactivity of the peroxy acid under the given experimental conditions; therefore, ethanol was used to increase the yield of monoepoxides.[30] This procedure was applied

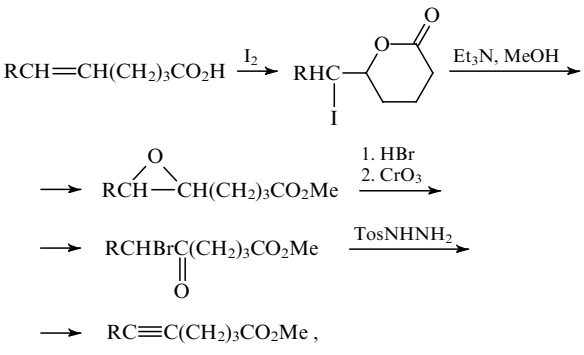
Table 1. The values of molar radioactivities (α) of labelled unsaturated fatty acids.[a]

Starting compound	α /PBq mol^{-1}	Ref.
Liquid phase isotope exchange		
Methyl oleate	0.146	28
Methyl linoleate	0.025	28
Methyl dihomo-γ-linolenate	0.034	28
Methyl arachidonate	0.092	28
Selective hydrogenation with gaseous tritium		
8,11,14-Triyne analogue of methyl dihomo-γ-linolenate	5.6	29
5,8,11,14-Tetrayne analogue of methyl arachidonate	7.4	29
5,8,11,14,17-Pentayne analogue of methyl thymnodonate	9.2	29
5-Acetylenic analogue of methyl arachidonate	1.3	16
14-Acetylenic analogue of methyl arachidonate	1.2	16
5-Acetylenic analogue of methyl thymnodonate	1.9	16
4-Acetylenic analogue of methyl docosahexaenoate	1.8	17

Note: In liquid-phase isotope exchange, the label is incorporated by isotope exchange of hydrogen atoms for tritium atoms; in hydrogenation with gaseous tritium, the label is incorporated owing to the reduction of triple bonds to double bonds.

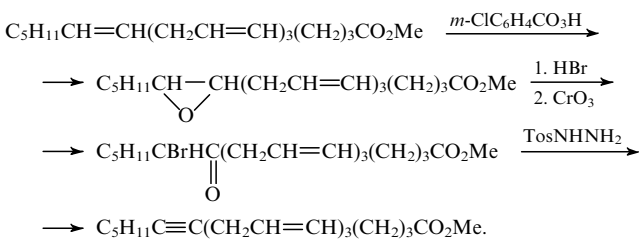
[a] Free fatty acids were obtained by alkaline hydrolysis of their methyl esters.

Scheme 1

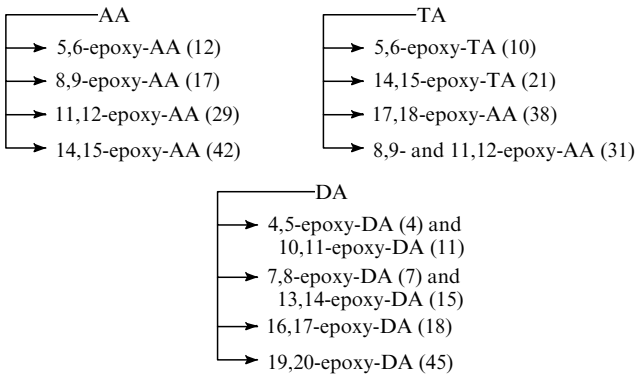


$R = C_5H_{11}CH=CH(CH_2CH=CH)_nCH_2$ (n = 2, 3).

Scheme 2



Scheme 3



The figures in parentheses indicate the percentage of the monoepoxide with respect to the total monoepoxide content in the reaction mixture.

for the preparation of monoepoxides from arachidonic (AA), thymnodonic (TA) and docosahexaenoic acids (DA) (Scheme 3).

Acetylenic analogues of eicosapolyenoic acids, *viz*., the 8,11,14-triyne analogue of methyl homo-γ-linolenate, the 5,8,11,14-tetrayne analogue of methyl arachidonate and the 5,8,11,14,17-pentayne analogue of methyl thymnodonate were synthesised by purely chemical methods (Scheme 4).

The reduction of triple bonds to double bonds in the atmosphere of gaseous tritium was used to prepare highly labelled polyenoic fatty acids (see Table 1). In order to increase the selectivity of hydrogenation of the corresponding acetylenic analogues, one had to modify palladium catalysts with lead diacetate, pyridine and quinoline. However, the yield of tritium-labelled alkenes did not exceed 10% – 40% even when the hydrogenation of one triple bond was performed according to the previously described procedures (*cf*. Ref. 5). Satisfactory yields were obtained with palladium on barium sulfate as a catalyst, which had been treated consecutively with methanolic solutions of lead diacetate [Pd : Pb = 1 : (0.5 – 1.0) mg mg^{-1}] and sodium borohydride [Pd : $NaBH_4$ = 50 : (0.4 – 4.0) mg mg^{-1}]. A 4 : 1 heptane – dioxane mixture was used as a solvent. The catalyst : substrate ratio was (2 – 6) : 1.[29] Under these conditions, the yield of the target products with a molar radioactivity of 1.2 – 1.8 PBq mol^{-1} per selectively reduced triple bond was 89% – 99% after 25 – 35 min (see Table 1).

It is necessary to emphasise that the reaction conditions were optimised not only for the labelling of unsaturated fatty acids, but also for pheromones, attractants and lipid hormones the complete chemical synthesis of which includes selective hydrogenation of acetylenic precursors in the final stage.[31] This can be exemplified in the use of acetylenic precursors in the synthesis of the *Lepidoptera* pheromones (Table 2).

Thus, bombykol (molar radioactivity 1.85 PBq mol^{-1}) was synthesised from hexadec-(10*E*)-en-12-yn-1-ol (see Ref. 29); the abscisic acid analogue (molar radioactivity 1.21 PBq mol^{-1}) (see Ref. 29) was prepared from (2*Z*)-5-(4-*tert*-butyl-*trans*-1-hydroxycyclohexyl)-3-methylpent-2-en-4-ynoic acid; retinoic acid (molar radioactivity 1.75 PBq mol^{-1}) (see Ref. 20) was obtained from 11,12-dehydroretinoic acid. (*Z*)-[11,12-^{3}H]Hexadeca-1,11-dien-3-one, (*Z*)-[6,7-^{3}H]eicos-6-en-11-one and some other labelled pheromones[32] with a molar radioactivity of ~ 1.5 PBq mol^{-1} were obtained by selective hydrogenation of the acetylene bonds.

The use of the liquid-phase isotope exchange for labelling saturated fatty acids is not promising, since molar radioactivities of reaction products were of the order of several hundredths of PBq mol^{-1} (Table 3). However, in contrast with alkenes, the isotope exchange in saturated compounds is not accompanied by side reactions; therefore, investigations into the use of this method were carried out and some regularities were established. Thus it was found that the kinetic curves of the isotope exchange reaction reach the plateau rather rapidly. In this case, the degree of substitution of tritium for hydrogen is much smaller than could

Scheme 4

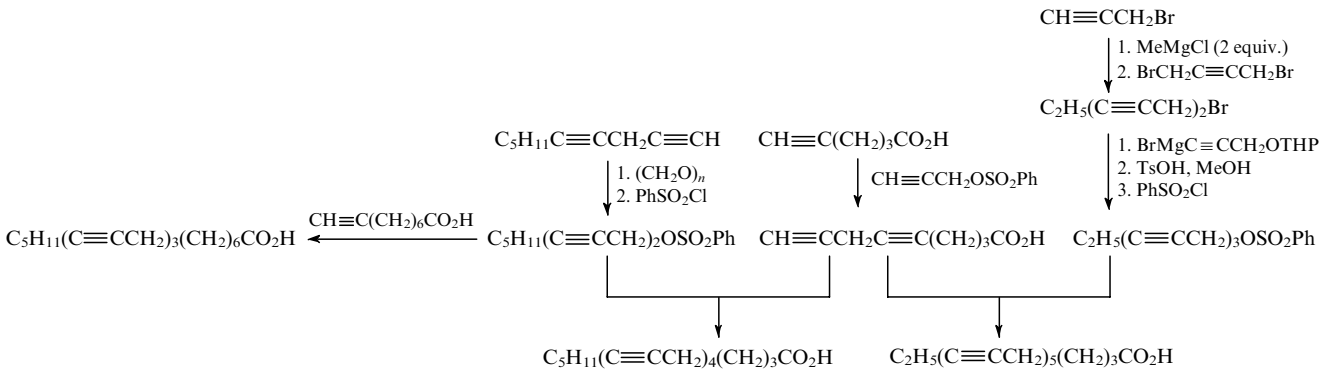


Table 2. Acetylenic precursors used in the synthesis of the pheromones of *Lepidoptera*.[31]

Acetylenic precursor	Pheromone component	Species
$Me(CH_2)_2C\equiv C(CH_2)_6CH_2OH$	(8*Z*)-dodecenyl acetate	*Grapholitha molesta*, *Cydia molesta*
$Me(CH_2)_9C\equiv C(CH_2)_4CHMe_2$	(7*Z*,8*Z*)-epoxy-2-methyloctadecane	*Porthetria dispar*
$Me(CH_2)_4C\equiv C(CH_2)_3CH(OH)(CH_2)_9Me$	(6*Z*)-heneicosen-11-one	*Orgyia pseudotsugata*
$Me(CH_2)_5C\equiv C(CH_2)_3C(O)(CH_2)_3Me$	(7*Z*)-eicosen-11-one	*Carposina niponensis*
$Me(CH_2)_3CH=CHC\equiv C(CH_2)_3CH_2OH$	(5*Z*,7*E*)-dodecadien-1-ol	*Dendrolimus spectabilis*
$MeCH_2C\equiv CCH=CH(CH_2)_5CH_2OH$	(7*E*,9*Z*)-dodecadienyl acetate	*Lobesia botrana*
$MeCH=CHCH_2C\equiv C(CH_2)_7CH_2OH$	(9*Z*,12*E*)-tetradecadienyl acetate	*Plodia interpunctella*, *Cadra cautella*
$Me(CH_2)_4C\equiv CCH_2CH=CH(CH_2)_2CH_2OH$	(4*E*,7*Z*)-tridecadienyl acetate	*Phthorimaea operculella*
$Me(CH_2)_3CH=CH(CH_2)_2C\equiv C(CH_2)_6OH$	(7*Z*,11*E*)-hexadecadienyl acetate	*Sitotroga cerealella*

Table 3. The values of molar radioactivities of saturated fatty acids.

Starting compound	α /PBq mol^{-1}	Ref.
Liquid-phase isotope exchange		
Methyl stearate	0.036	28
Methyl palmitate	0.021	28
Methyl myristate	0.014	28
Methyl laurate	0.010	28
Solid-phase isotope exchange		
Stearic acid	13.50	33
Palmitic acid	12.90	33
Myristic acid	12.10	33
Lauric acid	11.50	33
3-Hydroxypalmitic acid	9.00	34
6-Ketopalmitic acid	27.80	29
Methyl stearate	38.00	29
11-Chloroundecanoic acid	6.30	34
Cetyl alcohol	5.50	33
Hexadecane	17.50	33
Solid-phase dehalogenation		
11-Chloroundecanoic acid	4.20	34
Hydrogenation with gaseous tritium		
Palmitoleic acid	1.47	35
Undec-10-enoic acid	2.96	14
5-(3-Octynyloxy)pentanoic acid	3.89	36
12-Methoxydodec-1-ynoic acid	5.07	36

be expected from the equilibrium distribution of the tritium label. Besides, the kinetic curves of molar radioactivity have a complex shape, which is attributed to the different saturation of palladium with the hydrogen isotopes during the formation of the α-phase of palladium hydride and its subsequent transition into the β-phase.[28]

The high thermal stability of alkanes has made it possible to use solid-phase isotope exchange for introducing the label into saturated compounds (see Table 3).

Studies of solid-phase isotope exchange with stearic acid as a model compound in the presence of palladium and platinum catalysts supported on different carriers showed that this reaction proceeded with the greatest efficiency on 5% Pt/C at 210 °C (the catalyst : substrate ratio = 10 : 1) (Figs 1–4).‡

Similar investigations were carried out with stearic acid homologues. It was demonstrated that in this case, too, the optimal conditions for a solid-phase isotope exchange were as follows: 5% Pt/C, the catalyst : substrate ratio = 10 : 1, reaction time = 15 min. Other regularities were also established. First, the dependence of changes in the molar radioactivities of fatty acid homologues on the length of the carbon chain was practically linear, which points to the random labelling by this method. Second, the temperature at which the degree of isotope substitution was a maximum (T_{max}), was different for different analogues.

Acid	Lauric	Myristic	Palmitic	Stearic
M.p. /°C	44.3	58	64	70
T_{max} /°C	190	200	205–210	210

In all probability, this temperature depends on the phase transition temperature for representatives of the same homologous series (*e.g.*, fatty acids).

‡ In the study aimed at establishing the relative degree of the label incorporation, α_{rel}, the maximum molar radioactivity of the preparation was taken as 100%.

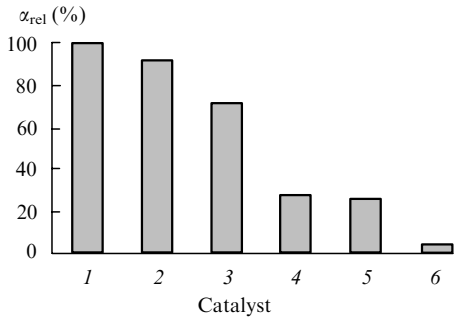


Figure 1. The dependence of the relative degree of the label incorporation into stearic acid on the nature of the catalyst.
Catalyst: (*1*) 5% Pt/C, (*2*) 10% Pd/C, (*3*) 10% Pd/$BaSO_4$, (*4*) 5% Pd/C, (*5*) 5% Pd/$BaSO_4$, (*6*) 5% Rh/C.

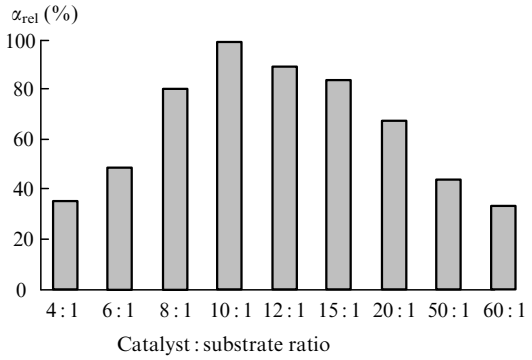


Figure 2. The dependence of the relative degree of the label incorporation into stearic acid on the catalyst : substrate (5% Pt/C) ratio.

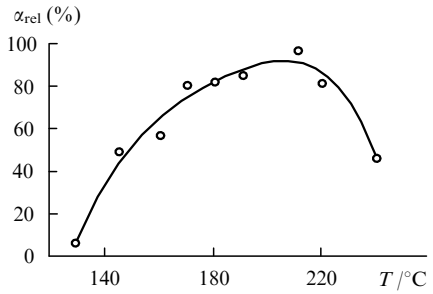


Figure 3. The dependence of the relative degree of the label incorporation into stearic acid on temperature (the catalyst is 5% Pt/C, the catalyst : substrate ratio is 10 : 1).

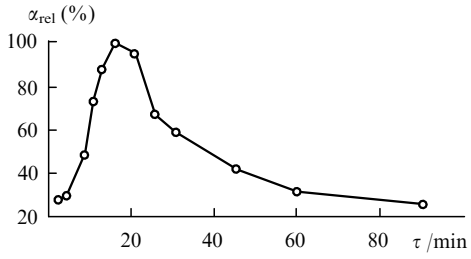


Figure 4. The kinetics of the label incorporation into stearic acid (the catalyst is 5% Pt/C, the catalyst : substrate ratio is 10 : 1, 210 °C).

It is evident that at certain temperatures the diffusion of these lipid molecules increases to such an extent that the rate of diffusion of the spill-over hydrogen no longer affects the rate of the overall reaction of isotope exchange. The efficiency of the isotope exchange decreases drastically after 10–15 min. The latter

circumstance can be rationalised as follows. The study of the valence state of hydrogen in hydrides of intermetallic compounds carried out by Semenenko *et al.*[37] has offered a new outlook on the problem of the valence state of hydrogen in systems of the $Pd-H_2$ type. Debates on this problem, which is of particular importance for hydride chemistry, have begun since the appearance of classical works of Ubbelhode,[38] who put forward a concept of a positively charged hydrogen atom on palladium, and of Gibb,[39] who proposed a model with a negatively charged hydride ion. Semenenko *et al.*[37] proposed and rationalised the concept according to which hydride dispersion is a result of a redox reaction associated with the formation of hydride ions, $H^{\delta-}$, at a hydrogen concentration (c_H) in the solid hydride exceeding a critical concentration ($c_{H_{crit}}$), which, in turn, depends on the redox potential of the reaction:

$$H_2 + M \leftrightarrow H^{\delta+} + M^{\delta-} \leftrightarrow H^{\delta'-} + M^{\delta'+},$$

where M is the metal template of the catalyst. According to this model, the hydrogen chemisorbed on the active centres on the catalyst surface donates some of its *s*-electrons to the metal conduction band. In this context, at low values of c_H the behaviour of the absorbed hydrogen is well described in terms of the Ubbelhode model[38] which entails high diffusion mobility of the $H^{\delta+}$ ions and an increase (or, at least, the lack of a decrease) in electric conductivity of the hydride phase in comparison with the original metal. The value of $c_{H_{crit}}$ corresponds to the concentration of the oxidising agent ($H^{\delta+}$ species) at which the latter oxidise the metal template and become electron density acceptors being converted into bulky, practically immobile $H^{\delta'-}$ ions. As a result, the metal conduction band is depleted in electrons. Obviously, at higher degrees of filling of cavities with $H^{\delta'-}$ ions having a fairly large effective volume, the electron deficiency on the metal ions forming a metal sublattice in the crystalline structure of the catalyst should increase which probably restricts its ability for reversible hydrogen absorption. In this connection, the concentration of the species (*e.g.*, of tritium in the case of labelled preparations) which can enter into exchange reactions drops drastically, eventually resulting in a practically complete cessation of the isotope exchange.

Approaches to the synthesis of fatty acids with high molar radioactivity were successfully used for the preparation of other highly labelled saturated lipids, *viz.,* fatty acid derivatives, fatty alcohols and alkanes (*e.g.*, methyl stearate, 3-hydroxypalmitic, 6-ketopalmitic and chloroundecanoic acids, cetyl alcohol and hexadecane) (Tables 4–6).[34] It was found that the isotope exchange in halogen-containing saturated acids occurs much easier than dehalogenation. The experimental values of molar radioactivities reached tens of PBq mol^{-1}, which makes it possible to conduct biological experiments of any kind including ligand–receptor binding studies. The nonspecific label distribution in the products obtained upon the isotope exchange is the only disadvantage of this method.

Table 4. The values of the relative degree of isotope exchange in 11-chloroundecanoic acid at various temperatures and on different catalysts (the catalyst : substrate ratio = 5 : 1, reaction time = 20 min).[34]

T /°C	5% Pt/C	10% Pd/C	5% Pd/C	10% Pd/$BaSO_4$	5% Rh/C
50	1	–	–	–	–
70	9	–	–	–	10
90	16	–	2	–	–
120	40	43	8	48	38
140	55	64	12	50	32
160	51	59	25	75	29
170	–	–	–	100	–
180	22	50	20	90	21
190	–	43	–	48	–
200	10	19	9	41	19
210	–	18	–	35	6
220	9	14	9	16	1

Table 5. The values of the relative degree of isotope exchange in cetyl alcohol at various temperatures and on different catalysts (the catalyst : substrate ratio = 6 : 1, reaction time = 15 min).[34]

T /°C	5% Pt/C	10% Pd/C	5% Pd/C	5% Rh/C
90	–	1	–	–
110	2	6	1	1
120	6	25	4	4
130	16	46	4	4
140	20	49	5	5
150	21	–	9	9
160	–	100	–	–
170	24	–	15	15
180	33	65	19	19
190	40	61	20	20
200	25	36	9	8
210	6	–	1	6
220	–	24	–	–

Table 6. The values of the relative degree of isotope exchange in hexadecane at various temperatures [the ratio 5% Pt/C : substrate = (6–10) : 1, reaction time = 15 min].[34]

Compound	Temperature /°C							
	90	140	160	180	190	200	210	230
Methyl stearate	2	12	44	–	–	–	100	30
6-Oxopalmitic acid	17	72	100	–	–	–	84	33
3-Hydroxypalmitic acid	1	81	100	89	–	–	–	–
Palmitic acid	2	44	60	76	–	80	100	30
Myristic acid	–	41	–	77	85	100	62	61
Lauric acid	–	52	–	86	100	96	78	69
Hexadecane	2	100	61	–	–	–	44	43

Hydrogenation of double bonds (see Table 3) is used in the synthesis of saturated fatty acids with a definite position of the label (its precursor is an accessible unsaturated acid).[4,29,35] Studies with [10,11-^{3}H]undecanoic acid obtained by this method, which is a component of a termite pheromone, have shown that it comes to the pheromone glands with food or is biosynthesised.

Among other methods used for the synthesis of labelled fatty acids, thermal activation of tritium deserves special mention. The use of this procedure for labelling of methyl 12-hydroxystearate (0.56 TBq mol^{-1}) was described by Shevchenko and Myasoedov.[4] The method consists in the application of a substance as a thin film onto the inner surface of a cylindrical vessel containing a tungsten filament placed along its axis. The system is evacuated, filled with tritium up to 0.67 Pa, cooled with liquid nitrogen and the tungsten filament is heated to 2000 K for 30 s. Tritium molecules dissociate, which significantly increases the degree of the isotope exchange.

[8L-^{3}H]- and [8D-^{3}H]Stearic acids[5] were obtained by conversion of methyl 8D(8L)-hydroxystearate into the corresponding tosylate, which was further reduced with lithium aluminium tritide (6.6 TBq mol^{-1}). Stearic alcohols formed were oxidised with CrO_3 to yield the target product (3.3 TBq mol^{-1}).

Labelled fatty acids find application in biological studies and in the synthesis of various derivatives and labelled oxylipins based on them. Thus, they can be used in studies of cholesterol esters biosynthesis[40] or in the synthesis of monoepoxides (see Schemes 1–3), which are the products of the epoxygenase pathway of the arachidonic acid cascade or other polyunsaturated fatty acids. The biological activity of these epoxides is widely known. They stimulate the *in vitro* release of somatostatin from

the median tubercle of hypothalamus and the secretion of insulin and glucagon from pancreatic islets, inhibit the vasopressin-stimulated release of H_2O in bladder tumours, suppress aggregation of platelets and neutrophils, enhance the release of calcium from dog aortic smooth muscle microsomes and increase the cytosolic levels of free calcium. Under the action of various enzymes, these epoxides are converted into other, more oxidised products, *e.g.*, vicinal diols, 20- and 19-hydroxy derivatives.

2. Fatty acid derivatives

a. Fatty acid esters

Glycerides. The high biological activity of glycerides can be exemplified in the properties of 2-arachidonoylglycerol, which is an intermediate in tri- and diglyceride metabolism and an alternative precursor of arachidonic acid manifesting cannabimimetic activity.[41] The use of a tritium-labelled analogue of this glyceride provided additional information about its pharmacological activity.

Labelled glycerides are usually obtained by condensation of the corresponding labelled fatty acids with glycerol.[4] Different approaches to the synthesis of triglycerides (TG) have been considered.[42] These lipids are formed upon heating of a mixture of glycerol and a fatty acid to 160–190 °C; the reaction proceeds more efficiently in the presence of toluenesulfonic acid with removal of water formed by distillation with xylene. Higher yields of TG are obtained in reactions of silver salts of fatty acids with 1,2,3-tribromopropane in boiling xylene and of acid chlorides or mixed anhydrides with glycerol. TG can also be synthesised under very mild conditions in the presence of dicyclohexylcarbodiimide and 4-dimethylaminopyridine. This procedure can also be used in the synthesis of the corresponding steroid esters. The use of this approach (the reaction was carried out in 1,2-dichloroethane; a solution of dicyclohexylcarbodiimide in benzene was added in several portions) resulted in labelled TG in more than 90% yield. Di- and monoglycerides can be prepared from triglycerides under the action of the corresponding lipases.[43] Thus 2-arachidonoylglycerol was prepared from triarachidonoylglycerol by treating it with *Rhizopus delemar* ('Seikagaku Kogyo', Japan) in 0.05 M AcONa–AcOH buffer (pH 5.6) containing 0.1 mmol litre^{-1} NaCl and 10 mmol litre^{-1} $CaCl_2$ with vigorous stirring for 60 min.[44]

Among other methods used for glyceride synthesis, special mention should be made of the reduction of dihydroxyacetone 1,3-derivatives with sodium borotritide and the isotope exchange with tritiated water. Molar radioactivities of TG obtained by this method were by one order of magnitude lower than those of the tritiated water employed.[4]

Studies with 2-[^{3}H]arachidonoylglycerol revealed that neuroblastoma N18TG2 cells contain nonspecific esterases. Hydrolysis of this monoglyceride was only partly (by less than 40%) inhibited by an excess of the unlabelled substrate, whereas the degree of selectivity of the esterase isolated from peripheral cells of the basophilic rat leukemia RBL-2H3 was much higher (74%). It was shown also that in contrast with 2-palmitoylglycerol, 2-linoleoylglycerol inhibited hydrolysis of 2-arachidonoylglycerol by intact cells as a result of which the concentration of 2-arachidonoylglycerol capable to activate the cannabinoid receptor increased considerably. The experimental results described by different authors[41,45–49] suggest that non-cannabimimetic monoglycerides enhance the effect of 2-arachidonoylglycerol by increasing the half-time of its conversion and increase binding of 2-arachidonoylglycerol to the cannabinoid receptor (4–8-fold).

Phospholipids. Formerly, phospholipids were considered exclusively as structural components of biological membranes which fulfilled the barrier function in respect of electrolytes and served as a matrix for membrane-bound enzymes and receptors. However, it has presently been recognised that phospholipids and the products of their enzymic and free-radical conversions also perform important cell regulatory functions, being substantial for the manifestation of catalytic activities of many enzymes. Besides, some phospholipids are endowed with hormone-like activities, play the role of platelet activation factors (PAF) (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine), accelerate wound healing (lysophosphatidic acid), *etc*. Hydrolysis of phosphoinositides and methylation of phosphatidylethanolamine are the key reactions in the transmission of intercellular signals.[50–52]

Table 7. The values of molar radioactivities of various phospholipids.

Compound	α /PBq mol^{-1}	Ref.
Hydrogenation with gaseous tritium		
1-*O*-[^{3}H]Alkyl-2-*O*-methyl-*sn*-glycero-3-phosphocholine	2.30	53
Lyso[^{3}H]phosphatidylcholine	0.14	28
[^{3}H]Phosphatidylinositol	0.50	28
1-*O*-[^{3}H]Palmitoyl-2-acetyl-*sn*-glycero-3-phosphocholine	1.85	54
1-*O*-[^{3}H]Stearoyl-*sn*-glycero-3-phosphocholine	0.48	11
[^{3}H]Phosphatidylcholine	0.54	28
1-{1′-Palmitoyl-2′-[9,10-^{3}H]stearoyl-*sn*-glycero-3′-phosphoryl}-*myo*-inositol-4-phosphate	0.39	29
Liquid-phase isotope exchange		
1,2-[^{3}H]Dipalmitoyl-*sn*-glycero-3-phosphocholine	0.02	29
Combination of chemical and enzymatic methods		
1,2-Dioleoyl-*sn*-[2-^{3}H]glycero-3-phosphoserine	0.93	55

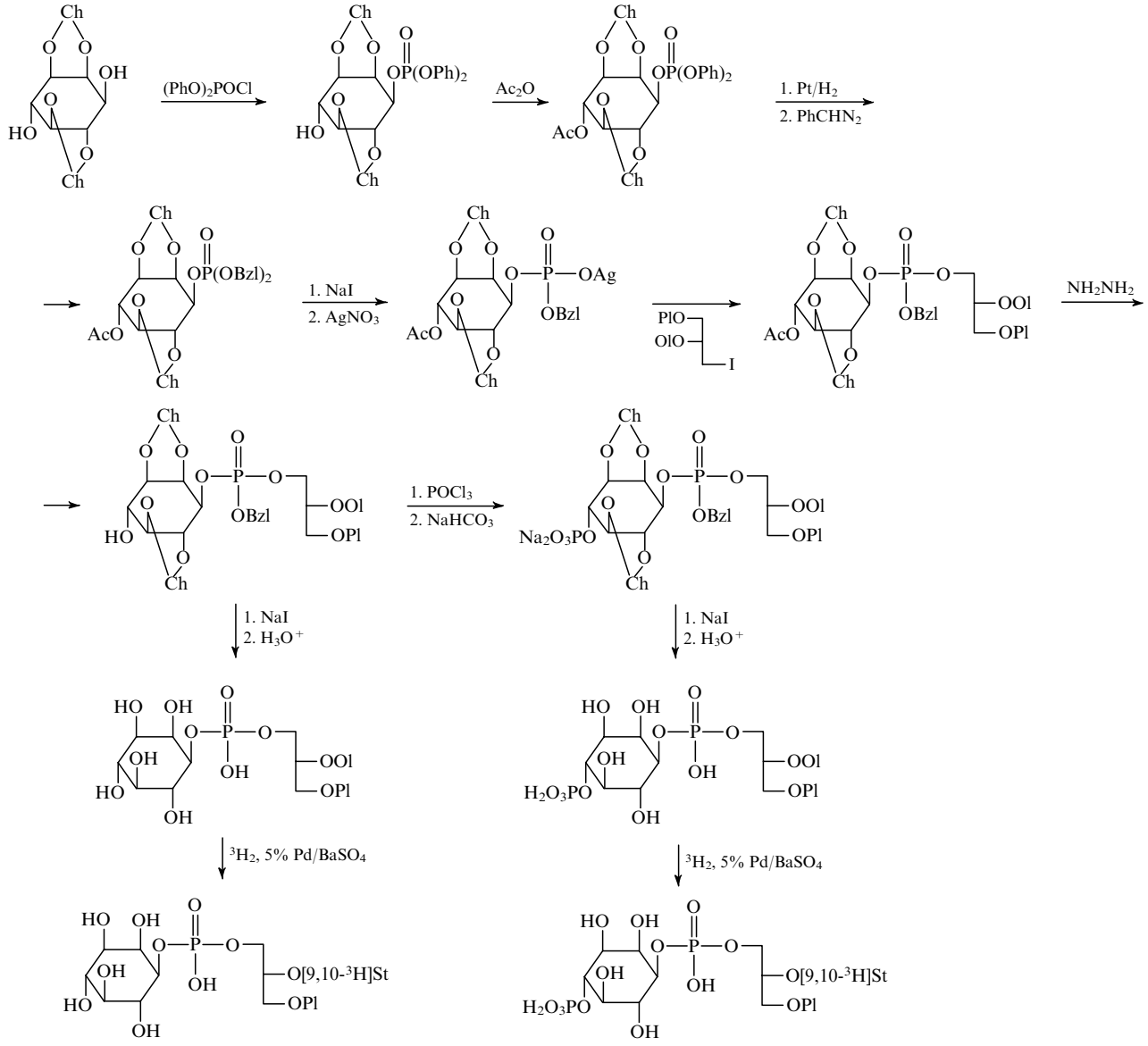
As a rule, the tritium label is introduced into phospholipids by hydrogenation of natural or synthetic unsaturated precursors (Table 7). The synthesis of tritium-labelled phosphatidylinositol and diphosphoinositide is shown in Scheme 5. Isotope exchange (Tables 7 and 8) and condensation of labelled fatty acids with the corresponding precursors as well as biological and enzymic methods and their combination are the most promising approaches.[4,55]

Unsaturated precursors were synthesised in order to prepare labelled phosphatidylinositol and diphosphoinositide.[56] Several steps of their synthesis including deacetylation with hydrazine hydrate, were identical. If the phosphatidylinositol was the target product, the protective groups were removed by anionic debenzylation and treatment with a cation-exchange resin (H^+-form). In

Table 8. The kinetics of liquid-phase isotope exchange between gaseous tritium and dipalmitoylphosphatidylcholine.[28]

Solvent	Time /min						
	15	30	45	60	90	150	180
Catalyst — 5% Pd/$BaSO_4$							
Chloroform	19	41	47	54	65	80	88
Chloroform : methanol (2 : 1)	33	50	56	73	78	97	100
Methanol	11	28	30	34	35	67	70
Lindlar catalyst							
Chloroform	1	2	2	2	2	3	4
Methanol	1	2	2	2	3	4	4
$(Ph_3P)_3RhCl$							
Chloroform	3	4	6	6	7	11	11

Scheme 5



Ch is cyclohexylidene, Pl — $C_{15}H_{31}CO$, Ol — $C_{17}H_{33}CO$, St — $C_{17}H_{35}CO$, Bzl is benzyl.

the synthesis of diphosphoinositide, inositol was phosphorylated at position 4 with an excess of phosphorus oxychloride in pyridine with subsequent deprotection. Hydrogenation of the unsaturated fatty acid residues in the *myo*-inositol derivatives in an atmosphere of gaseous tritium in the presence of a catalyst has led to highly labelled phospholipids.[29]

These conditions proved to be versatile and can be used in the synthesis of labelled sterol esters. Thus, the hydrogenation of cholesteryl oleate was used to prepare cholesteryl [9,10-^{3}H]-stearate[29] with a molar radioactivity of 1.98 PBq mol^{-1}. Condensation of [9,10-^{3}H]oleic acid (0.07 PBq mol^{-1}) with lysophosphatidylcholine in the presence of dicyclohexylcarbodiimide and L-dimethylaminopyridine (1 : 1 : 1 : 100) in ether (stirring for 20 h at room temperature) affords lecithin (yield 35% – 40%).[4]

The synthesis of phosphatidyl[^{3}H]inositol can serve as an example of a synthesis of tritium-labelled fatty acid derivatives by biological methods.[4] The starting compound, [2-^{3}H]inositol, was synthesised by solid-phase reduction[35] of a commercially available *scillo*-inosose (2,4,6,3,5-pentahydroxycyclohexanone).[35] In a search for optimum conditions for *scillo*-inosose reduction, 5% Pd/$CaCO_3$, 5% Rh/Al_2O_3, 5% Pd/$BaSO_4$, 5% Pt/C, 5% Pd/C were tested as catalysts. The best results were obtained on a rhodium catalyst (the catalyst : substrate ratio = 10 : 1) with heating to 150 °C for 15 min (the molar radioactivity of the labelled preparation was 0.56 PBq mol^{-1}, the yield was 25%). In addition, *scillo*-inosose with a molar radioactivity of 0.11 PBq mol^{-1} was isolated from the reaction mixture in ~ 10% yield.

The biosynthesis of the labelled phospholipid was performed by incubation of [2-^{3}H]inositol with a culture of *Kloeckera brevis* (ATCC 9774) (7 days, 25 °C). Subsequent extraction, chromatographic purification and desalting resulted in phosphatidyl[^{3}H]inositol, which contained 54% of the original label and had a molar radioactivity ~ 2 times as low as that of the original [2-^{3}H]inositol.

A combination of chemical and enzymic methods was used in the synthesis of 1,2-dioleoyl-*sn*-[2-^{3}H]glycero-3-phosphoserine.[55] The first step included the chemical synthesis of [2-^{3}H]glycerol; its incubation with glycerol kinase in the presence of ATP gave *sn*-[2-^{3}H]glycerol-3-phosphate (yield 98% – 99%). Boiling of the monotetraethylammonium *sn*-[2-^{3}H]glycerol-3-phosphate tetraethylammonium oleate and oleic acid anhydride (1 : 10 : 10) in dry carbon tetrachloride (3 h, 80 °C) afforded 1,2-dioleoyl-*sn*-[2-^{3}H]glycerol-3-phosphate in ~ 56% yield. The pyridinium salt of the latter was condensed with the benzhydryl ester of *N*-Boc-

serine in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (1 : 3.2 : 6.4) in dry pyridine (24 h, 30 °C). Deprotection under acidic conditions and chromatographic purification yielded 1,2-dioleoyl-*sn*-[2-^{3}H]glycero-3-phosphoserine (total yield 12%, molar radioactivity 0.93 PBq mol^{-1}).

Labelled phospholipids play an important role in present-day biological studies. Thus in a study of the role of PAF in the regulation of cellular activity and cell-to-cell contacts, its labelled analogue was used in order to establish the effect of different antagonists blocking PAF activity at the level of PAF receptors on cell activation.[51] A synthetic lipid antagonist CV-3988 inhibited the binding of the labelled phospholipid to platelets and suppressed the secretion and aggregation of platelets (0.1 – 0.15 μmol litre^{-1}).[57] The binding of this phospholipid to PAF receptors is known to trigger a cascade of biological reactions which result in the signal transfer into the cell and its subsequent activation.[51] It has been shown[58] that 5 s after treatment of platelets with PAF, phosphatidylinositol-4,5-diphosphate undergoes hydrolysis to inositol-1,4,5-triphosphate. This hydrolysis is catalysed by membrane-bound phospholipase C. It was found also that the antagonists inhibit both the binding of labelled PAF to platelets and the formation of inositoltriphosphate in platelets.[59] The role of inositoltriphosphates in the regulation of the Ca^{2+} level in cell cytoplasm is immense, which may account for the pronounced effect of phospholipids on vital activity of cells in general.[50]

b. Fatty acid amides

Ethanolamides of fatty acids represent highly active endogenous bioregulators. Arachidonic acid ethanolamides, which has been named later anandamide, was isolated from porcine brain in the early 1990's.[60] This compound proved to be an endogenous ligand of cannabinoid receptors, *viz*., it competitively inhibited the specific binding of cannabinoids to receptors and mimicked their biological effects.[60–67] Ethanolamides of other polyenoic fatty acids were isolated from mammalian brain more recently.[68,69] These compounds also bind specifically to cannabinoid receptors. It is established now that mammalian tissues manifest synthase and amidase activities responsible for the synthesis and inactivation of anandamide.[70–77] Tritium-labelled ethanolamides of fatty acids were used in studies of their metabolism, the activity of enzymes of the arachidonic acid cascade in the oxidation of these fatty acid derivatives and the effects of these labelled compounds on functioning of the arachidonic acid cascade in different animal and plant cells.

Ethanolamides can be labelled at both the acyl and ethanolamide fragments. Highly labelled fatty acids can be synthesised by selective hydrogenation in an atmosphere of gaseous tritium, while labelling of ethanolamine can only be done by solid-phase methods.[78,79] Glycolic acid nitrile proved to be a promising starting compound, and 5% Pt/C, 5% Rh/C, 5% Pd/C, 5% Rh/Al_2O_3 and 5% Rh/$CaCO_3$ are effective catalysts. The highest yields were obtained with palladium catalysts; the molar radioactivity was higher on rhodium catalysts. The latter were used in the synthesis of labelled ethanolamine with a molar radioactivity of 1.04 – 1.48 PBq mol^{-1}, but the yields of target products were so insignificant that preparative syntheses of the labelled compound could be carried out only in the presence of 5% Pd/C. On platinum catalysts, dehydrogenation predominantly occurs.

The data summarised in Table 9 suggest that T = 100 – 120 °C is the optimum temperature. Under these conditions, the labelled product was obtained in 10% – 20% yield and had a molar radioactivity of 0.15 – 0.22 PBq mol^{-1}.[78] Isobutyl chloroformate[78] or *N,N′*-diisopropylcarbodiimide[79] were used for condensation of fatty acids with ethanolamine (Table 10).

Studies on kinetics of oxidation of labelled anandamide and thymnodonic acid ethanolamide with soybean 15-lipoxygenase and on their possible oxidation in mouse splenocytes can serve as examples of application of these labelled compounds in biological studies.[80] It was found that the values of Michaelis constants (K_m) and V_{max} for these compounds were equal to 3.2 and 4.9 μmol litre^{-1} and to 0.11 and 0.15 μmol l^{-1} s^{-1}, respectively. These values are of the same order of magnitude as those for arachidonic acid.[79,81] Experiments have shown that mouse splenocytes contain an anandamide-metabolising system. Splenocytes are related to cells continuously secreting 12-HETE and other eicosanoids. Anandamide-induced changes in the concentration of these products modulate functions of lymphocytes and other eicosanoid-controlled immune cells.

Table 9. The values of the relative degree of the label incorporation and yield of ethanolamine in the hydrogenation of glycolonitrile for different times and at different temperatures (the ratio 5% Pd/C : substrate = 5 : 1).

T /°C (20 min)	Degree of label incorporation (α_{rel}) (%)	Reaction time /min (100 °C)	Yield (%)
20	10	5	6
50	31	10	7
70	55	15	11
90	66	20	15
100	100	30	15
120	100	40	13
140	65	50	12
160	32	60	8
180	12	75	6
190	6	90	3

Table 10. The values of molar radioactivities of ethanolamides.

Compound	α /PBq mol^{-1}	Ref.
N-Arachidonoyl[^{3}H]ethanolamine	0.012	79
N-Dihomo-γ-linolenoyl[^{3}H]ethanolamine	0.019	79
N-Eicosa-11,14-dienoyl[^{3}H]ethanolamine	0.019	79
N-Eicosa-11-enoyl[^{3}H]ethanolamine	0.023	79
N-Palmitoyl[^{3}H]ethanolamine	0.012	79
N-γ-Linolenoyl[^{3}H]ethanolamine	0.026	79
N-[^{3}H]Arachidonoylethanolamine	4.4	78
N-Arachidonoyl[^{3}H]ethanolamine	0.18	78
N-Thymnodonoyl[^{3}H]ethanolamine	0.182	78
N-Docosahexaenoyl[^{3}H]ethanolamine	0.182	78

Sphingolipids pertain to the class of lipid molecules with different chemical structures and functional activities. Sphingenin (sphingosine) is the most common sphingosine base of human and animal cell sphingolipids. The sphingenin molecule contains two chiral carbon atoms and can exist in four stereoisomeric forms. Natural sphingenin represents a D-*erythro*-isomer. N-Acylation of the amino group of sphingenin with fatty acids results in ceramides, the precursors of more complex sphingolipids (sphingomyelin and glycosphingolipids).

Until the 1980's, the effect of the structure of the acyl residues of these lipids on their biological activity had been virtually neglected.[82] However, studies carried out in the late 1980's provided evidence that sphingenin inhibited cell differentiation,[83] while ceramides activated it.[84–87] More recent studies have shown that the biological properties of the fatty acid residues of ceramides depend on their lengths. Thus it was found that C_{18}-ceramide does not inhibit lymphocyte proliferation (in contrast to the short-chain ceramides C_2 and C_6),[88] whereas hydroxy acids present in natural ceramides strongly suppressed the induction of apoptosis.[89] The activation of protein phosphatase with ceramides also depends on the length of the acyl chain.[90] This can be ascribed to the hydrophobic interactions between the hydrocarbon chains of sphingosine and the fatty acid which depend on the structure of both the sphingosine chain (length, the presence (sphingenin) or the absence (sphinganin) of a double bond) and the fatty acid residue (length, the presence or the

absence of double bonds, their number, position, the presence of hydroxy groups, *etc.*). Such interactions may attenuate the differences in the effector activities of ceramides and dihydroceramides. Thus investigations into the mechanism of action of ceramides on target cells revealed that dihydroceramides, unlike the corresponding ceramides, inhibited (rather than stimulated) the activity of protein phosphatases,[90,91] but had no effect on the activity of the protein kinase C isoform [92] and phospholipase D,[93] which are inhibited by ceramides. However, in certain tests *N*-acetylsphingenin and *N*-acetylsphinganin manifested identical activities.

Radioactive labelling of sphingolipids can be performed in different ways, such as by condensation of the corresponding aminodiol with labelled fatty acids, by enzymic or chemical oxidation of hydroxy groups of sphingolipids with subsequent reduction with sodium or potassium borotritides, biosynthesis of labelled gangliosides starting from labelled *N*-acetyl-D-mannosamine and hydrogenation of the corresponding unsaturated precursors with gaseous tritium (Table 11).

The latter method was used for the synthesis of labelled gangliosides, sphingomyelin and ceramides. It was found [4,5] that the ^{3}H-label adds preferentially to the carbon atoms of multiple bonds. If liquid-phase isotope exchange is used for the labelling of unsaturated fatty acids, the label is predominantly incorporated into the allylic positions and into the double bonds. In phospholipids, the greater part of the label is localised in the fatty acid residues, whereas in the case of sphingolipids it is detected in both fatty acid and sphingosine moieties (Table 12)

Labelling of cerebrosides on the Lindlar catalyst results in the label incorporation into the fatty acid moiety, while on 5% Pd/$BaSO_4$ as a catalyst, it incorporates into the sphingosine moiety. This can be explained by the fact that the molecules of these compounds contain several polar groups (carboxy, hydroxy, amino, *etc.*), which compete with one another for the active centres on the catalyst's surface.[28] Presumably, the adsorption of the fatty acid residue on the Lindlar catalyst is more preferential than the adsorption of sphingosine bases. This fact determines the distribution of the tritium label.

It should be noted that chemical methods can be used for the synthesis of compounds with a predetermined position of the label.[95,96] Complete chemical synthesis was used to obtain labelled D-*erythro*- and L-*threo*-sphingosines (Scheme 6).

Starting from galactose, azidosphingosines were prepared; the allylic hydroxy group was oxidised with 2,3-dichloro-5,6-dicyanobenzoquinone with subsequent reduction of the resulting ketone with sodium borotritide.[95]

An attractive approach was proposed by Toyokuni *et al.*[96] Natural sphingenin was used as the starting compound; its primary hydroxy group was oxidised with CrO_3 (the Collins reagent) after protection of the amino group and the allylic hydroxy group. The reduction of the aldehyde formed with sodium borotritide and deprotection gave [1-^{3}H]sphingenin with the natural configuration.

Labelled sphingolipids were used to study the interaction of gangliosides with low density lipoproteins (using tritium-labelled ganglioside G_{M3}) [97] and the role of gangliosides in the reception and penetration of the influenza virus into the cell.[98] In the latter

Table 11. The values of molar radioactivities of sphingolipids.

Compound	α /PBq mol^{-1}	Ref.
Chemical methods		
N-Oleoylsphinganin [a]	7.78	94
(2*S*,3*R*,4*E*)-2-Amino[3-^{3}H]dodec-4-ene-1,3-diol [b]	0.019	95
(2*S*,3*S*,4*E*)-2-Amino[3-^{3}H]dodec-4-ene-1,3-diol [b]	0.034	95
(2*S*,3*R*,4*E*)-2-Amino[3-^{3}H]octadec-4-ene-1,3-diol [b]	0.020	95
(2*S*,3*S*,4*E*)-2-Amino[3-^{3}H]octadec-4-ene-1,3-diol [b]	0.036	95
(2*S*,3*R*,4*E*)-2-Amino[1-^{3}H]octadec-4-ene-1,3-diol [b]	0.004	96
Hydrogenation with gaseous tritium		
Sphingomyelin	0.218	28
Ganglioside		
$G_{D1\alpha}$	0.115	28
$G_{D2\beta}$	0.137	28
G_{M1}	0.196	28
G_{M2}	0.496	28
G_{M3}	0.014	28
G_1	0.159	28
G_2	0.107	28
Galactosyl ceramide [c]	1.94	4
Galactosyl ceramide [d]	0.895	4

Note: Brain and liver gangliosides are designated according to Svennerholm ($G_{D1\alpha}$, $G_{D2\beta}$, G_{M1}, G_{M2}, G_{M3}); sea urchin (*Strongylocentrotus intermedius*) gangliosides are designated as G_1 (*N*-glycolylneuraminosyl-(α,2 → 6)-glucosyl-(1 → 8)-*N*-glycolylneuraminosyl-(2 → 6)-glucosyl-(1 →1)-ceramide) and G_2 (8-sulfo-*N*-glycolylneuraminosyl-(α,2 → 6)-glucosyl-(1 → 8)-*N*-glycolylneuraminosyl-(2 → 6)-glucosyl-(1 → 1)-ceramide).

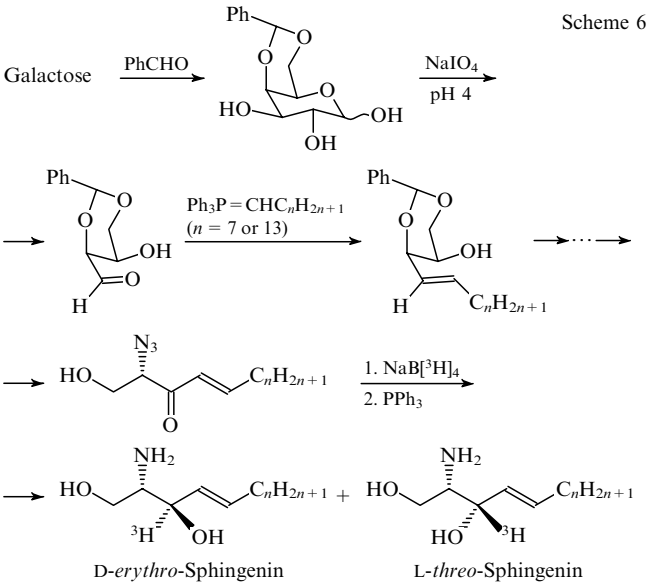
[a] The solid-phase method with subseqent condensation with oleic acid was used for introducing the label into sphinganin; [b] Sodium borotritide was used. [c] 5% Pd/$BaSO_4$ was used as a catalyst. [d] The Lindlar catalyst was used.

Table 12. The distribution of the tritium label in labelled glycosphingolipids after a catalytic reaction with gaseous tritium in solution.[28]

Compound	Catalyst	Tritium activity /PBq mol^{-1}			
		fatty acids	hydroxy fatty acids	sphingosine bases	polar fragments
Galactosyl ceramide	5% Pd/$BaSO_4$	0.368 (19)	0.291 (15)	1.163 (60)	0.116 (6)
Galactosyl ceramide	Lindlar catalyst	0.260 (29)	0.340 (38)	0.296 (33)	–
Ganglioside					
$G_{D1\alpha}$	5% Pd/$BaSO_4$	0.009 (8)	–	0.094 (82)	0.012 (10)
G_2	$(Ph_3P)_3RhCl$	0.086 (80)	–	0.020 (19)	0.001 (1)
G_1	5% Pd/$BaSO_4$	0.065 (33)	–	0.116 (59)	0.016 (8)
G_{M2}	5% Pd/$BaSO_4$	0.298 (60)	–	0.169 (34)	0.030 (6)
Sphingomyelin	Lindlar catalyst	0.140 (64)	–	0.076 (35)	0.002 (1)

Note: The percentage of the activity of a given moiety relative to the activity of the original molecule is given in parentheses.

Scheme 6

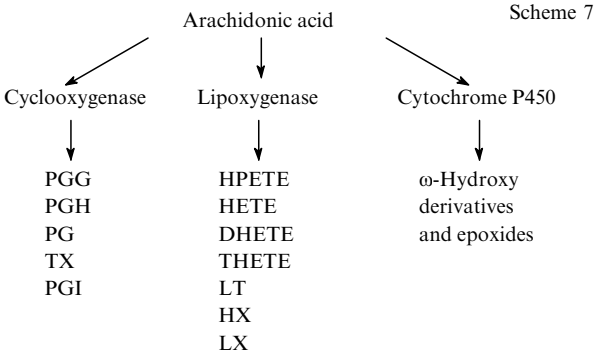


case, Ehrlich ascites carcinoma cells, which are efficient receptors of the influenza virus, were employed. The viral adsorption decreased drastically after treatment of cells with *Vibrio cholerae* neuraminidase which points to the fact that sialo-containing compounds (gangliosides) are specific receptors of the virus on the cell surface. Indeed, gangliosides G_{M1}, $G_{D1\alpha}$ and $G_{T1\beta}$ restored the viral adsorption by cells. Based on the experimental data, it was concluded that: (1) the oligosaccharide chain of the ganglioside, Galβ1 → 3 GalNAcβ1 → 4(NeuAcα2 → 3)Galβ1 → 4 Glc, is the 'minimum' structural element providing the adsorption of the influenza virus by the gangliosides; (2) the gangliosides probably take part in both binding and penetration of the virus into the cell; one receptor site binds ~ 100 ganglioside molecules involved in the reception.

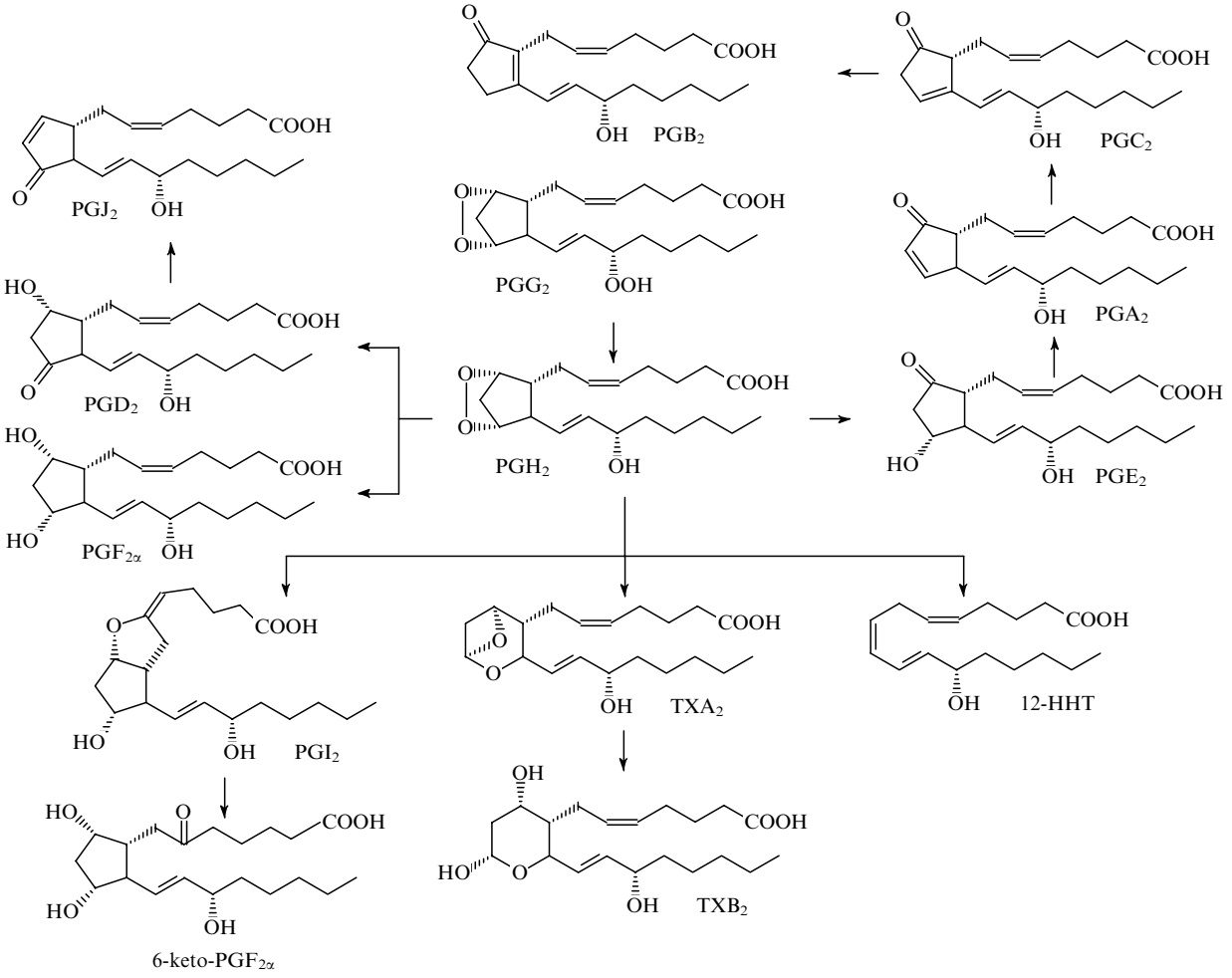
III. Oxylipins

Oxylipins comprise eicosanoids and eicosanoid-like compounds with shortened or lengthened carbon chains, which are synthesised, for the most part, by cyclooxygenase, lipoxygenase and cytochrome P450 pathways.[5, 99] The main pathways of arachidonic acid metabolism resulting in oxylipins, *e.g.*, prostaglandins (PG), thromboxanes (TX), leukotrienes (LT), hydroxyeicosatetraenoic acids (HETE), hydroperoxyeicosatetraenoic acids (HPETE), lipoxins (LX), hepoxylins (HX), epoxytrienoic acids (EET), *etc.*, are depicted in Scheme 7. It is of note that the scheme demonstrates exclusively transformations of arachidonic acid and its derivatives; however, other eicosapolyenoic acids can also be used as substrates in this reaction.

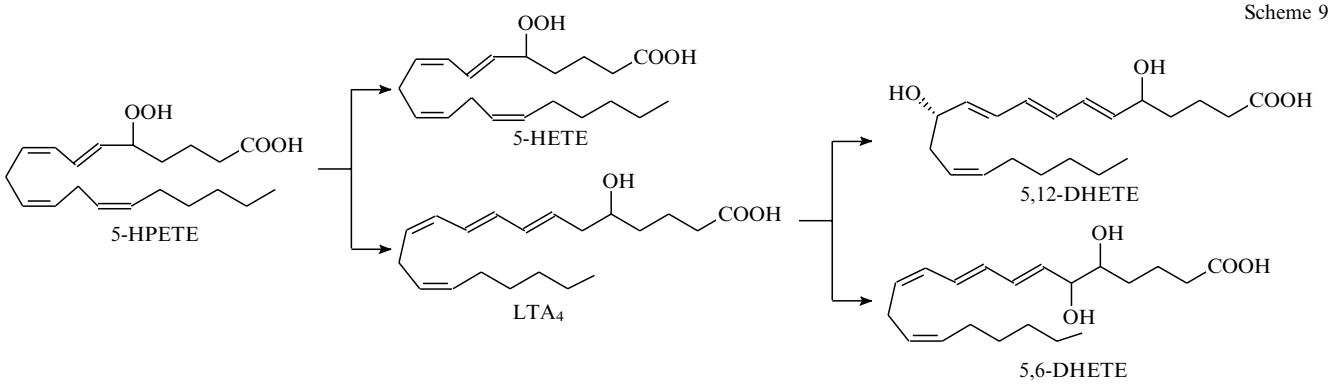
Scheme 7



Scheme 8



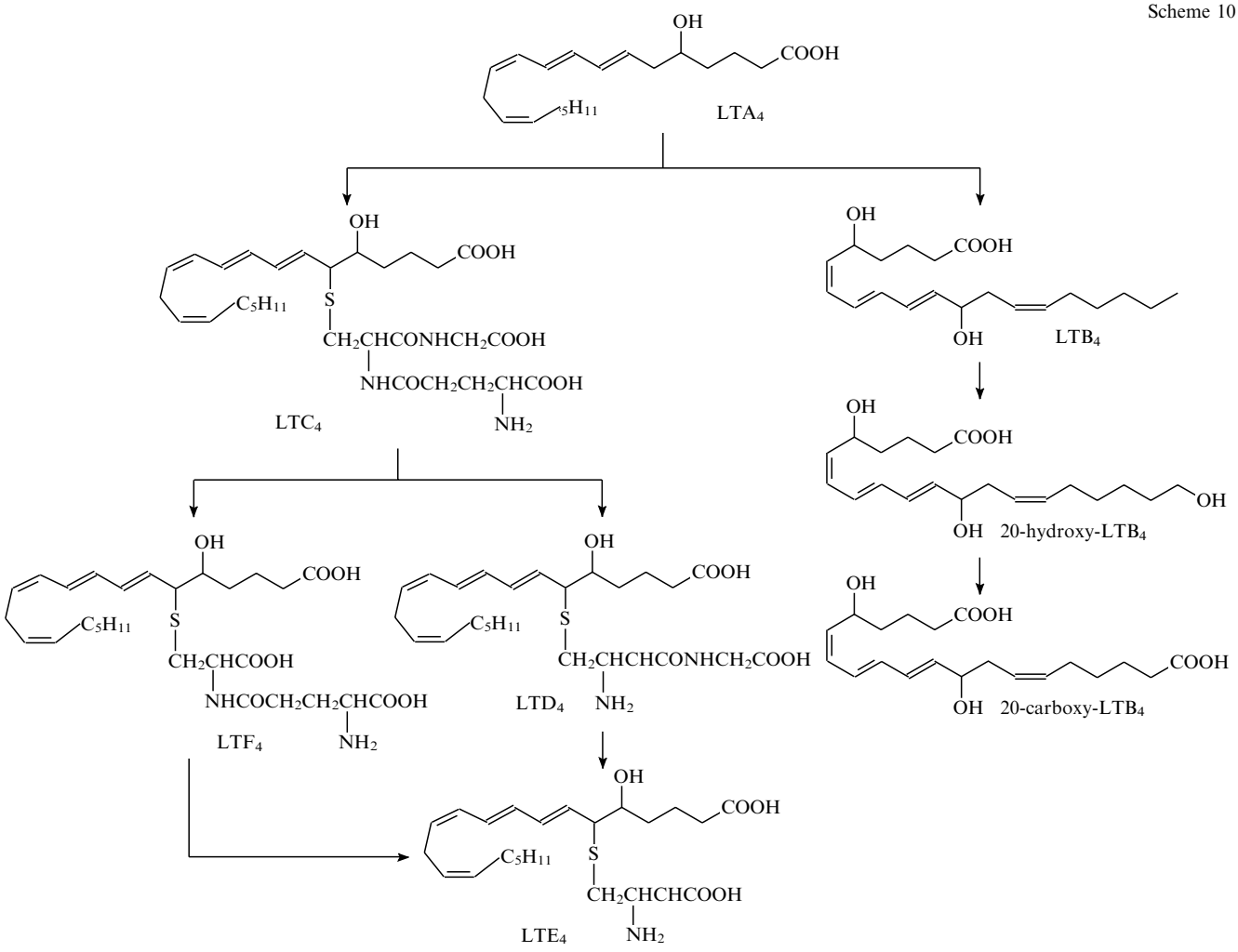
Scheme 9



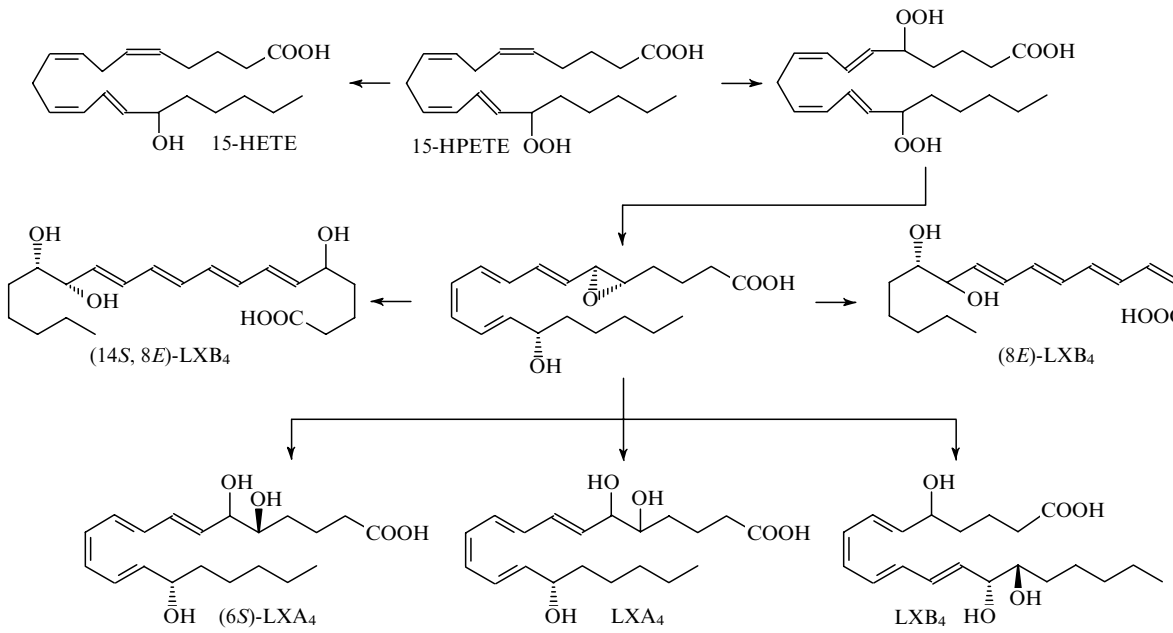
Under the action of enzymes, arachidonic acid yields: (1) PG, TX and prostacyclines (PGI), (2) hydroxy acids (LT, HX, LX); (3) epoxy and ω-hydroxy derivatives of fatty acids. Scheme 8 depicts preparation of PG and TX from prostaglandin G_2, Schemes 9 and 10, of hydroxy acids and LT from 5-HPETE, Schemes 11 and 12, of hydroxy acids, LX and HX from 12- and 15-HPETE. The synthesis of ω-hydroxy acids and epoxides is exemplified in oxidation of arachidonic acid in the presence of cytochromes P450 (Scheme 13).

The biological and pharmacological effects of these compounds are extremely diverse. Thus lipoxins control the activity of killer cells and manifest spasmogenic activity;[100] hepoxylins are the releasing factors in insulin secretion by pancreatic cells and regulate the transmembrane transfer of calcium;[101] leukotrienes are mediators of hypersensitivity (allergy, anaphylaxis).[102] Prostaglandins have a wide application as medicinal drugs.[103] However, despite the diversity of oxylipins and the broad spectrum of their biological activities, it is the continuous interaction of these compounds with one another that provides for the normal course of vitally important reactions occurring in the organism. The validity of this concept was confirmed in the studies of the role of arachidonic acid metabolites in the maintenance of the normal functioning of hemostasis in mammals.[104]There exist at least two ways of maintaining the necessary level of arachidonic acid in a hemostatic system, *viz*., from cell phospholipids and from plasma low density lipoproteins. The metabolites of polyunsaturated fatty acids (prostacyclin, 13-hydroxyoctadecadienoic acid, 6-keto-PGE_1, PGE_1, HETE and HPETE, TXA_2, PGE_2, $PGF_{2\alpha}$,

Scheme 10



Scheme 11

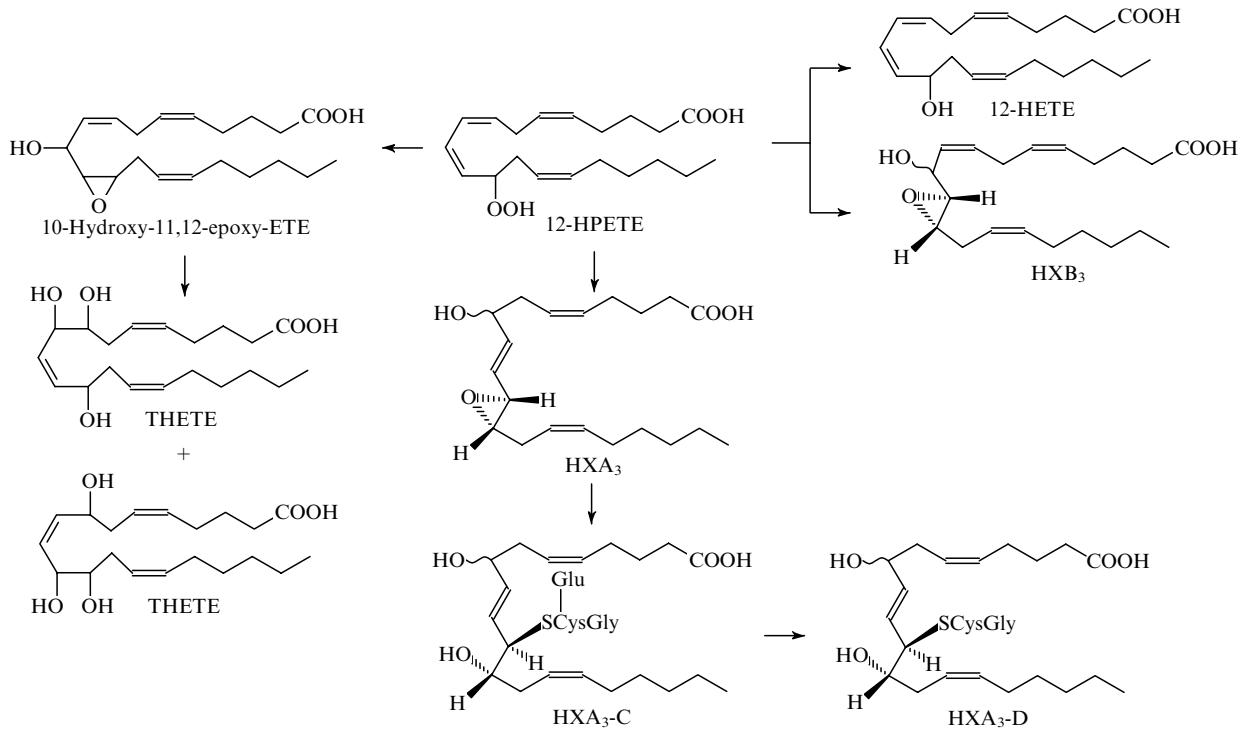


$PGD_{2\beta}$, LTA_4, LTB_4) participate in the regulation of practically all units of the blood coagulating system as well as in reactions of the plasma component of hemostasis by influencing the fibrinolytic potential of blood and hemodynamic conditions. Thus, the urgency of investigations of these compounds is dictated by the fact that their effect on enzymic oxidation of polyenoic fatty acids is the central link in the mechanism of action of the majority of bioeffector lipids.

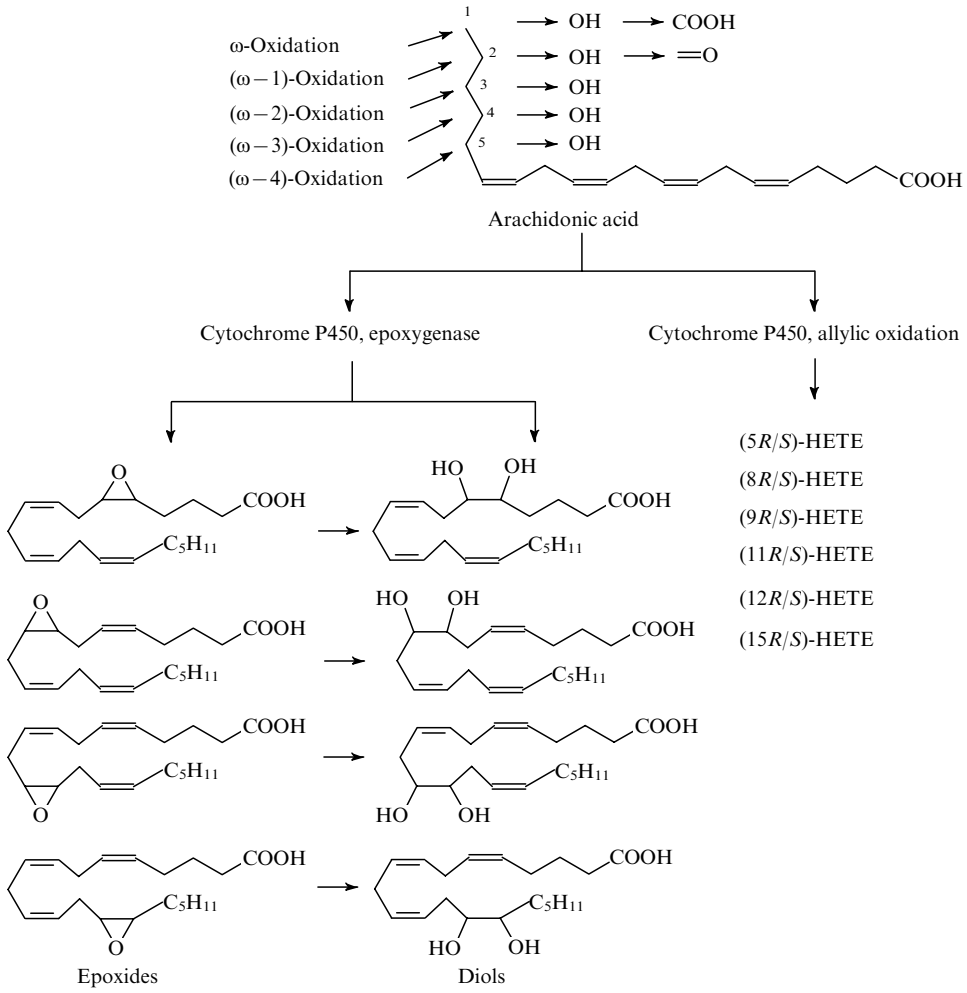
The main investigations in the field of oxylipin with established structure are aimed at the study of their biosynthesis and metabolism. Labelled oxylipins have played a prominent role in these studies. For this reason, a part of the present review which is devoted to the synthesis of labelled oxylipins by enzymic methods can be regarded as an illustrative example of their application in biological studies. In addition, studies of metabolic conversions of oxylipins also consider their properties, *e.g.,* solubility, penetrability through biological membranes, *etc.* Labelled analogues turned out to be a helpful tool in pharmacokinetic studies of the first medicinal drugs (as a rule, natural prostaglandins and their synthetic analogues). Besides, they are indispensable components in the elaboration of highly sensitive methods for determination of oxylipins in biological samples and in reception studies (interaction with protein molecules).

Radioactive labels can be introduced into eicosanoids by several routes. Isotope exchange or chemical methods are used for labelling synthetic derivatives of oxylipins, where non-labelled prostaglandins are used as precursors or where the yields of the target products obtained by biological methods are negligibly

Scheme 12



Scheme 13

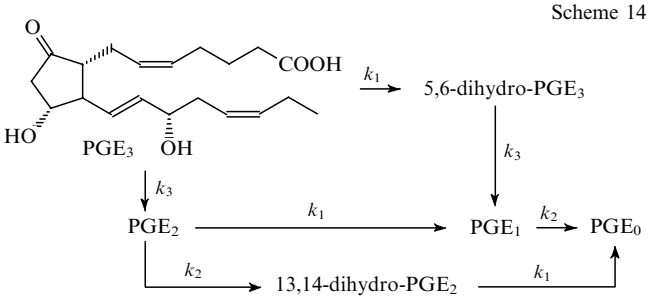


small (*e.g.*, the synthesis of epoxy compounds, prostacyclin, *etc.*), as well as for interconversion of labelled compounds.

The isotope exchange and chemical methods are indispensable tools in the synthesis of labelled eicosanoids having a non-natural structure, *e.g.*, (17*S*)-17-methyl-20-[11-^{3}H]homoisocarbacycline,[105] fluoro prostaglandins,[106] *etc*. These methods were used to obtain fluorinated compounds with molar radioactivities varying from 0.09 to 1.1 PBq mol^{-1} (Table 13).

Selective hydrogenation of prostaglandins of series 2 and 3 (*e.g.*, of PGE_3 to PGE_2 and PGE_1, Scheme 14) with gaseous tritium [107] afforded labelled preparations with molar radioactivities of 1.5–2.9 PBq mol^{-1} (see Table 13).

Scheme 14



In the latter method, the results of calculations in assumption that the rates of all reactions are described by first-order equations showed good agreement with experimental results. In this case, the ratio of hydrogenation rate constants (k_1, k_2, k_3) were 2 : 1 : 4 for the reaction in acetone and 2 : 1 : 8 for the reaction in a benzene : acetone mixture. Obviously, for the synthesis of labelled PGE_2 from PGE_3 the benzene : acetone mixture is more preferable; under these conditions, the yields of [17,18-^{3}H]PGE_2 and [5,6,17,18-^{3}H]PGE_1 were 40%–45% and 10%–15%, respectively.

The use of chemical methods for the synthesis of labelled compounds requires a detailed analysis of their chemical properties. Thus it is known [5] that prostaglandins E are converted into PGA in acidic media, into PGB in alkaline media and into PGF after treatment with borohydrides. The latter in turn can serve as a precursor in the synthesis of prostacyclin or 6-keto-$PGF_{1\alpha}$ (see Table 13). Chemical methods can also be used for the synthesis of labelled methyl esters, *e.g.,* LTA_4 and 8-epi-$PGF_{2\alpha}$ (Scheme 15).[108, 109, 110]

The choice of catalysts for selective hydrogenation with gaseous tritium was carried out as described in Section II.1. The best results were obtained with palladium catalysts poisoned with lead diacetate. In the case of more labile compounds, triethylamine was added to a solvent in order to minimise the formation of side products.

Selective hydrogenation with gaseous tritium can be used in the synthesis of other oxylipins, *e.g.*, [5,6,8,9,14,15-^{3}H]hepoxylins B_3 (Scheme 16).[19]

Enzymic methods with the use of labelled eicosapolyenoic acids as the starting substrates (see Scheme 7) are the most popular methods for the synthesis of labelled eicosanoids. It is impossible to mention all of the currently known data concerning this problem in a single review; therefore, we shall confine our description to the consideration of a few examples illustrating the versatility of this approach.[111] Thus the use of only one enzyme, *e.g.,* PG synthase, which catalyses the transformation of eicosa-

Table 13. The values of molar radioactivities of oxylipins obtained by hydrogenation with gaseous tritium, isotope exchange and chemical methods.

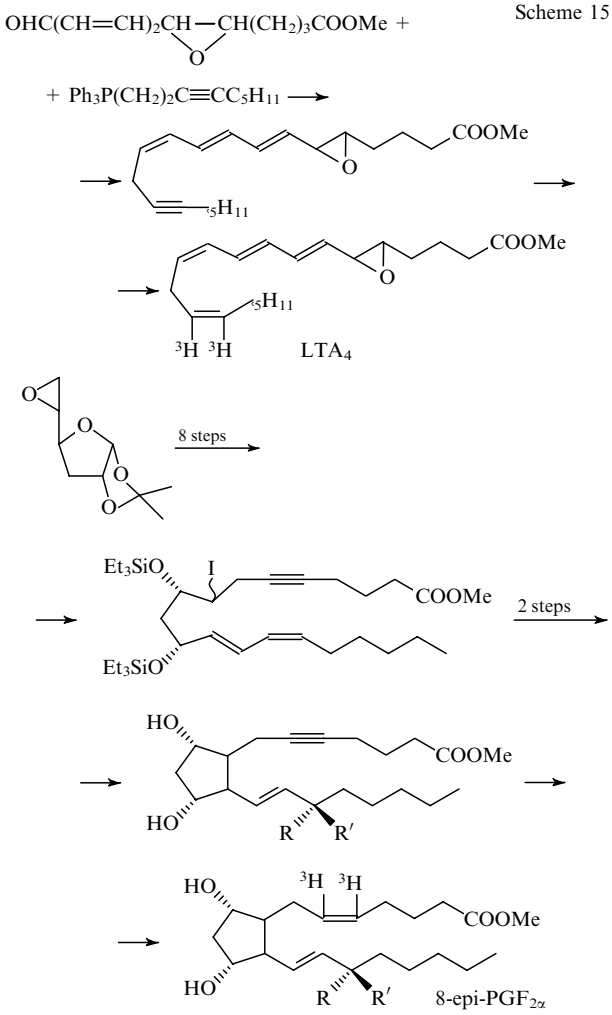
Compound	α /PBq mol^{-1}	Ref.
Hydrogenation with gaseous tritium		
[13,14-^{3}H]Leukotriene A_4 ME	1.48 – 1.67	108
(15*S*)-8-epi-[5,6-^{3}H]$PGF_{2\alpha}$	1.85 – 1.93	108
(15*R*)-8-epi-[5,6-^{3}H]$PGF_{2\alpha}$	1.85 – 1.93	108
[5,6,8,9,14,15-^{3}H]HXB_3 ME	2 – 4	19
[17,18-^{3}H]PGE_2	1.5 – 1.6	29
[5,6,17,18-^{3}H]PGE_1	2.8 – 2.9	29
[5,6-^{3}H]PGE_1	1.8 – 2.0	29
Fluoro-15-deoxy-[5,6-^{3}H]PGE_1	0.9 – 1.1	29
11-Deoxy-[5,6,10,11-^{3}H]PGE_1	2.6 – 3.0	29
Liquid-phase isotope exchange		
15-Fluoro-11,15-dideoxy-[G-^{3}H]PGE_1 ME	0.13	29
15-Fluoro-15-deoxy-[G-^{3}H]$PGF_{2\alpha}$ ME	0.09	29
5-Iodo-15-fluoro-15-deoxy-[G-^{3}H]PGI_1 ME	0.05	29
Chemical methods		
(17*S*)-17-methyl-20-[11-^{3}H]homoiso-carbacycline [a]	0.68	105
[8,11,12,14,15-^{3}H]PGA_1 [b]	4.1	29
[5,6,8,11,12,14,15-^{3}H]PGA_2 [b]	5.2	29
[5,6,8,11,12,14,15,17,18-^{3}H]PGA_3 [b]	7.3	29
[11,14,15-^{3}H]PGB_1 [b]	2.9	29
[5,6,11,14,15-^{3}H]PGB_2 [b]	3.7	29
[5,6,11,14,15,17,18-^{3}H]PGB_3 [b]	5.7	29
[8,11,12,14,15-^{3}H]$PGF_{1\alpha}$ [b]	4.6	29
[5,6,8,11,12,14,15-^{3}H]$PGF_{2\alpha}$ [b]	5.8	29
[5,6,8,11,12,14,15,17,18-^{3}H]$PGF_{3\alpha}$ [b]	7.3	29
[8,11,12,14,15-^{3}H]$PGF_{1\beta}$ [b]	4.6	29
[5,6,8,11,12,14,15-^{3}H]$PGF_{2\beta}$ [b]	5.8	29
[5,6,8,11,12,14,15,17,18-^{3}H]$PGF_{3\beta}$ [b]	7.3	29
[5,8,11,12,14,15-^{3}H]PGI_2 ME [c]	5.0	29
6-Keto-[5,8,11,12,14,15-^{3}H]$PGF_{1\alpha}$ME [d]	4.9	29

Note: ME is methyl ester. [a] Sodium borotritide with a molar radioactivity of 3.04 PBq mol^{-1} was used. [b] Prepared from [^{3}H]PGE. [c] Prepared from [^{3}H]$PGF_{2\alpha}$ME; [d] Prepared from [^{3}H]PGI_2ME.

polyenoic acids into PGH (its half-life in aqueous media is ~ 5 min), made it possible to obtain a variety of labelled products, such as PDG, PGE, PGF_α and 12-hydroxyheptadecatrienoic acid (HHT) (see Scheme 8). The yield of labelled PGH and, consequently, of its degradation products depends on the concentrations of the eicosapolyenoic acid and PG synthase. Under optimum conditions, the yield of [5,6,8,9,11,12,-14,15-^{3}H]PGH_2 was 70%; those of [5,6,8,9,11,12,14,15,-17,18-^{3}H]PGH_3, [5,6,8,9,11,12-^{3}H]HHT, [5,6,8,9,11,12,14,-15-^{3}H]$PGF_{2\alpha}$, [5,6,8,9,11,12,14,15,17,18-^{3}H]$PGF_{3\alpha}$ and [5,6,8,9,-12,14,15,17,18-^{3}H]PGD_3 were 45%, 35%, 63%, 27% and 25%, respectively. The ratio of products of non-enzymic degradation of PGH_2 in different buffer systems is variable (Table 14).

The use of two-enzyme systems has made it possible to find conditions for the predominant formation of only one reaction product and to synthesise compounds which cannot be obtained by spontaneous degradation of PGH. In this case, PG synthase was supplemented with the enzymes specifically converting labile [^{3}H]PGH into [^{3}H]PGE, [^{3}H]PGD, labelled thromboxane and [^{3}H]HHT, *viz*., PGE synthase for the synthesis of [^{3}H]PGE, PGD synthase for the synthesis of [^{3}H]PGD and TX synthase for the synthesis of [^{3}H]TXB and [^{3}H]HHT. In a study with individual enzymes, conditions for eicosanoid biosynthesis were optimised by varying the concentrations of each enzyme and their ratios.[112] All the preparations used in this study retained their activities

Scheme 15



R = OH, R′ = H; R = H, R′ = OH.

upon storage (6 months at -60 °C). Even highly labile PGE synthase retained up to 80% of activity for 12 days at -60 °C. Inactivation of enzymes (PG synthase, 12-lipoxygenase, TX synthase) in the course of biosynthesis was taken into consideration using the following kinetic equation:

$$\frac{de}{ds} = \frac{1}{V},$$

where e is the concentration of the active enzyme species, s is the substrate concentration and V is the dimensionless parameter reflecting the number of the enzyme turnovers up to complete inactivation and depending on the chemical nature of the

Scheme 16

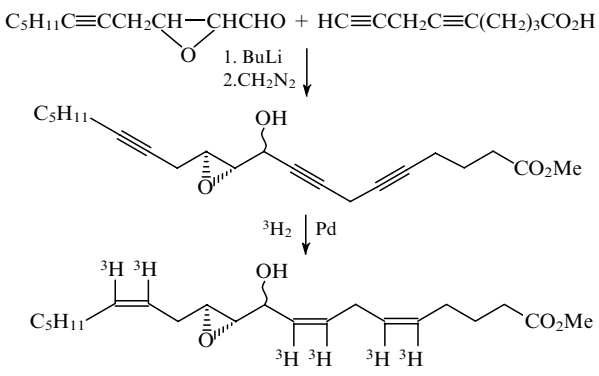


Table 14. The composition of PGH_2 nonenzymic degradation products obtained in a PG synthase reaction in various buffers.[111]

Buffer	Buffer concentration /mmol litre^{-1}	Prostaglandin content (%)		
		PGD_2	PGE_2	$PGF_{2\alpha}$
Tris buffer, pH 9.5	10	12	23	65
Potassium phosphate buffer, pH 7.4	50	19	39	42
Potassium phosphate buffer, pH 7.4 [a]	50	29	26	45

[a] After 30-s incubation, hydrochloric acid was added to the reaction mixture to pH 3.0 and the incubation was continued (1 h, 25 °C).

substrate. The experimental values of V for the PG synthase-catalysed conversions of arachidonic and thymnodonic acids[110] differed more than 10-fold.

Quantitative calculations carried out with the use of a mathematical model and experiments with two-enzyme systems have shown[112] that the maximum yields of target products can only be obtained if the substrate is gradually added to the reaction mixture containing both enzymes. Under these conditions, the yields of target eicosanoids are restricted by enzyme inactivation in the course of the reaction. It was also found that the efficiency of a two-enzyme system can be significantly increased if some specified initial concentrations of the enzymes and the substrate are used.

Thus, the strategy of enzymic synthesis of labelled eicosanoids is based on the use of artificial two-enzyme systems and can be formulated as follows.[112] First, enzyme preparations must be purified to the maximum degree, since ballast proteins cause strong adsorption of the original labelled eicosapolyenoic acids, make them inaccessible for PG synthase and provoke undesirable conversions of PGH (Table 15).

Second, the enzyme : substrate ratio should be an optimum, *e.g.,* 747 and 63 nmoles of arachidonic and thymnodonic acids, respectively, should be taken per unit of PG synthase activity.

Third, high yields of target products can be obtained at high concentrations of PGH-converting enzymes. Thus in the case of PGE_2, a fourfold increase in the concentration of PGE synthase caused a 1.9-fold increase in the eicosanoid yield; in the case of PGD_2, a threefold increase in the concentration of PGD synthase increased the eicosanoid yield twofold, whereas in the case of TXB2, a fivefold increase in the concentration of TX synthase

Table 15. The effect of the degree of purification of enzyme preparations on the eicosanoid content in the reaction mixture.[111]

Enzyme preparation	Yield (%)
Synthesis of PGE_2	
Ram vesicular gland microsomes	50
Partially purified PG and PGE synthases	80
Synthesis of PGE_3	
Ram vesicular gland microsomes	30
Partially purified PG and PGE synthases	45
Synthesis of TXB_2	
Ram vesicular gland microsomes and human platelets	15
Partially purified PG and thromboxane synthases	80
Synthesis of 12-HETE	
Human platelet microsomes	35
Partially purified 12-lipoxygenase	50

Table 16. The yields of eicosanoids in two-enzyme systems with simultaneous (I) and separate (II) addition of PG synthase and PGH-converting enzyme to the reaction mixture.[111]

Eicosanoid	Yield (%)	
	I	II
PGD_2	23	33
PGD_3	17	25
TXB_2	13	24
TXB_3	10	18

increased the yield of the target product 2.4-fold. Probably, this is associated with the necessity for creation of conditions where the rates of enzymic conversions of PGH will markedly exceed the rate of its spontaneous degradation.

Fourth, the procedure of conducting the biosynthesis plays a very important role. The yield of the target product can be increased 1.5–2-fold by separate addition of the enzymes to the reaction mixture (Table 16). The optimum time of incubation of an eicosapolyenoic acid with PG synthase prior to the addition of the second enzyme is 1.5–2 min. Within this period, the accumulation of PGH is a maximum, *i.e.*, the rate of its formation by the PG synthase reaction coincides with the rate of its nonenzymic degradation.

Fifth, the temperature dependence of the yields of these eicosanoids in two-enzyme synthesis has a broad optimum (20–25 °C). This implies that the optimal yields of labelled eicosanoids can be attained at room temperature.

This strategy was successfully used in the synthesis of various highly labelled oxylipids (Table 17).

Considerable interest recently arose in the studies of functioning of various enzymic subsystems under heterophasic conditions.[112] This interest is due to the fact that the catalytic activity and stability of many enzymes under reaction conditions can be significantly increased. Thus a search for the ways aimed at increasing the 'productivity' of enzymes involved in prostaglandin synthesis, which undergo irreversible inactivation in the course of the reaction, has demonstrated high efficiency of macroheterogenous systems (water-in-octane and octane-in-water). It was found that the activities of the enzymes of eicosanoid synthesis (PG synthase, PGE synthase, TX synthase, 12-lipoxygenase) were retained in all heterophasic systems under study. The highest values of activity, V, for the PG synthase

Table 17. The values of molar radioactivities of various oxylipins obtained by enzymic methods.

Compound	α /PBq mol^{-1}	Ref.
[8,11,12,14,15-^{3}H]PGE_1	4.65	29
[5,6,8,11,12,14,15-^{3}H]PGE_2	5.90	29
[5,6,8,9,11,12,14,15-^{3}H]$PGF_{2\alpha}$	6.10	29
[5,6,8,9,12,14,15-^{3}H]PGD_2	5.98	29
[5,6,8,9,11,12,14,15-^{3}H]TXB_2	5.27	29
[5,6,8,11,12,14,15,17,18-^{3}H]PGE_3	7.35	29
[5,6,8,9,12,14,15-^{3}H]PGD_2	4.44	113
13,14-Dihydro-15-keto-[^{3}H]PGD_2 [a]	—	113
[9α,11β-^{3}H]PGF_2 [a]	—	113
[15-^{3}H]HETE	6.20	114
[5,15-^{3}H]DHETE	5.10	114
[8,15-^{3}H]DHETE	6.00	114
12-[^{3}H]HPETE	5.26	115
[12-^{3}H]HETE	5.26	115
[15-^{3}H]HPETE	5.37	115
[15-^{3}H]HETE	5.37	115

[a] Prepared from [5,6,8,9,12,14,15-^{3}H]PGD_2.

reaction were observed with octane-in-water emulsions stabilised with native microsomal membranes. It was also found that if water : octane : lyophilised membranes ratios were close to those at which the system is stratified, the catalytic activity of PG synthase increased nearly 10-fold in comparison with systems containing no organic solvents. Moreover, the main advantage of this approach to the synthesis of labelled preparations is that in stabilised emulsions the labelled eicosapolyenoic acid is predominantly accumulated in the organic solvent, whereas the oxidation products (*e.g.*, PGE_2) are detected only in the aqueous phase. As a result, the negative effect of radiolysis on the formation of target labelled eicosanoids is reduced and the reaction products, the enzyme and the nonconsumed substrate are readily separated by centrifugation.

In addition to these methods for the synthesis of labelled oxylipins, special mention should be made of the possibility of synthesis of certain labelled prostaglandins by enzymic modification of other labelled prostaglandins. The use of chemical methods is coupled with the formation of stereoisomers and the necessity to perform additional steps for protection of functional groups with similar reactivities. Enzymic syntheses of prostaglandins starting from other prostaglandins as substrates are devoid of these drawbacks. Thus 15-keto-$PGF_{2\alpha}$, 13,14-dihydro-15-keto-$PGF_{2\alpha}$, PGE_2, 15-keto-PGE_2, 13,14-dihydro-15-keto-PGE_2 and 9α,11β-PGF_2 were synthesised from [^{3}H]$PGF_{2\alpha}$ and [^{3}H]PGD_2 using 9- and 15-hydroxyprostaglandin dehydrogenases, PGF synthase, *etc*., isolated from various sources (see Table 17).[111–113]

Labelled 15-HETE, 5,15-DHETE, 8,15-DHETE and 12-HETE were synthesised in up to 60%–65%, 20%–25%, 10%–15% and 21%–51% yields, respectively, using enzymes of the lipoxygenase pathway of eicosapolyenoic acid metabolism (*e.g.*, 15- and 12-lipoxygenase) (see Schemes 11 and 12),.

The optimum conditions for these reactions were selected in the studies of the dependence of the yields of target products on the presence of appropriate additives in the reaction mixture as well as on the substrate : enzyme ratio, substrate and enzyme concentrations, degree of enzyme purification, *etc*.[111, 114, 115]

An analysis of the data presented in Table 18 suggests that if the 12-lipoxygenase reaction is conducted in the presence of certain reducing agents, the yield of 12-HETE increases considerably. However, the use of enzymes is not always successful. Thus labelled leukotriene B_4 was synthesised in but 0.1% yield using enzymes of the lipoxygenase pathway of eicosapolyenoic acid metabolism,[29] whereas the yields of lipoxins[116] were negligibly small. These examples demonstrate that the use of enzymic methods demands a careful selection of reaction conditions and a thorough analysis of factors decreasing the yield of target products.

Table 18. The yields of 12-HETE from [^{3}H]arachidonic acid depending on the nature of the reducing agents.[111]

Reducing agent	Yield (%)
–	34
Dithiothreitol	51
Glutathione	49
L-Cysteine	44
L-Tryptophan	36
Aniline	34
L-Adrenaline	32
L-Methionine	32
NADH	31
Phenol	29

IV. Steroids

The fundamental problem of a relationship between the structure of biologically active compounds and their activities is still very urgent. Its solution would make it possible to use both natural and modified steroids as medicinal drugs. It is known that steroid hormones fulfil several functions in the organism, each being mediated by a specific receptor. Studies of the mechanism of steroid–receptor interaction shed light on what fragments of the steroid molecule are responsible for one or another of its biological function. This knowledge will allow the minimising of potential side effects of natural steroids used as medicinal drugs, on the one hand, and influencing their particular biological function by modifying natural steroid molecules, on the other hand.[22]

Tritium-labelled steroids possessing estrogenic, androgenic and anabolic activities are necessary for estimating the biological characteristics of pharmacological preparations used in studies on reproduction and increase of the productivity of animals. Labelled analogues of steroid hormones are used in investigation of various metabolic processes in normal and pathological states, in diagnostics of diseases and quantitation of steroids in various biological fluids.[117] The topicality of synthesis of labelled steroids is connected with the application of their natural precursors in the treatment of various diseases, such as atherosclerosis, asthma, rheumatism, eczemas, allergies, polyarthritis, gout, radiation disease, diabetes mellitus, *etc*.

The label is introduced into steroids by various routes. Let us consider the methods used in the synthesis of labelled cholesterol as an example.

The data presented in Tables 19–21 suggest that dehalogenation, solid-phase isotope exchange and isotope exchange with

Table 19. The values of molar radioactivities of cholesterol obtained by isotope exchange and by chemical methods.

Method of labelling	α /TBq mol^{-1}	Ref.
Using recoil atoms ^{6}Li(n,α)^{3}H	0.0022	3, 4, 8, 118
Isotope exchange:		
incubation in an atmosphere of gaseous tritium (the Wiltzbach method)	74.1	3, 4, 8, 118
activation of the reaction with an electric charge	185.2	3, 4, 8, 118
addition of a carrier	0.0445	3, 4, 8, 118
microwave irradiation	0.9207	3, 4, 8, 118
liquid-phase isotope exchange	1.7	4
solid-phase isotope exchange	997.3	119
isotope exchange with tritiated water	218.0	121
Chemical methods:		
reduction with metal tritides	785.2	118
dehalogenation of the 7-halogeno derivative	370.0	120
dehalogenation of the 2,2-dihalogeno derivative	1129.6	118
hydrogenolysis of the 7,7-disulfur-containing derivative	300.1	122

Table 20. The relative degree of isotope exchange (*a*) and yields of steroids depending on duration of the reaction.[121]

τ /h	Progesterone		Estriol		Cholesterol	
	a	yield (%)	*a*	yield (%)	*a*	yield (%)
2	48	95	22	95	27	95
4	75	90	48	85	69	85
6	97	85	81	75	84	80
10	100	70	100	60	100	65
24	98	20	95	18	95	15
50	100	10	96	10	94	10

Table 21. The values of molar radioactivities of steroids in isotope exchange with tritiated water.[121]

Compound	α /PBq mol^{-1}
Progesterone	0.248
Estradiol	0.400
Cortisol	0.218
Methylandrosta-1,4-dien-17α-ol	0.200
Methylandrost-5-ene-3β,17α-diol	0.226

tritiated water are the most promising methods.[118–122] The label can successfully be introduced into androstanes, pregnanes and some other steroids by a modified Wiltzbach procedure (see Ref. 123). Labelled steroids can also be prepared by biological methods,[120] hydrogenation and selective hydrogenation of the corresponding precursors[124–127] in combination with other chemical methods (Table 22).[129–133]

Dehalogenation allows one to prepare stereospecifically labelled steroids. Molar radioactivities of the thus synthesised [7-^{3}H]cholesterol, [7-^{3}H]pregnenolone and [7-^{3}H]dehydroepiandrosterone amount to 0.37 PBq mol^{-1}, and those of aromatic 2,4-dibromosteroids, to 1.1 PBq mol^{-1}.[118–120]

Biological methods entail the conversion of labelled steroids into other steroids. Thus [16-^{3}H]aldosterone (molar radioactivity 0.08 PBq mol^{-1}) was prepared from [16-^{3}H]progesterone (0.5 PBq mol^{-1}) using bovine adrenal cortex enzymes, and [16-^{3}H]cortisol (molar radioactivity 0.07 PBq mol^{-1}) was obtained from [16-^{3}H]corticosterone with a molar radioactivity of 0.14 PBq mol^{-1}.[120]

It is known that the reactivity of double bonds of unsaturated steroid hormones decreases in the following order: 1,2 > 6,7 > 16,17 > 4,5. By changing the reaction conditions (solvents, catalysts, *etc.*), one can perform selective hydrogenation of a definite bond and obtain the target steroid with high molar radioactivity.[118,120] This method seems to be the most versatile for radioactive labelling of steroids; this results in steroids with high molar radioactivities.[130,133] The syntheses of 17α-ethynyl-19-nor[9,11-^{3}H]testosterone and 18-methyl-17α-ethynyl-17β-hydroxy[14α,15α-^{3}H]estr-4-en-3-one are shown in Schemes 17 and 18.

These schemes provide illustrative examples of the potentialities of chemical approaches to the synthesis of complex labelled compounds which are very labile under conditions of treatment with gaseous tritium in the presence of catalysts. If biological studies require labelled saturated steroids, solid-phase hydrogenation is the method of choice. In this case, the molar activity of steroids increases owing to the isotope exchange (Table 23).[120]

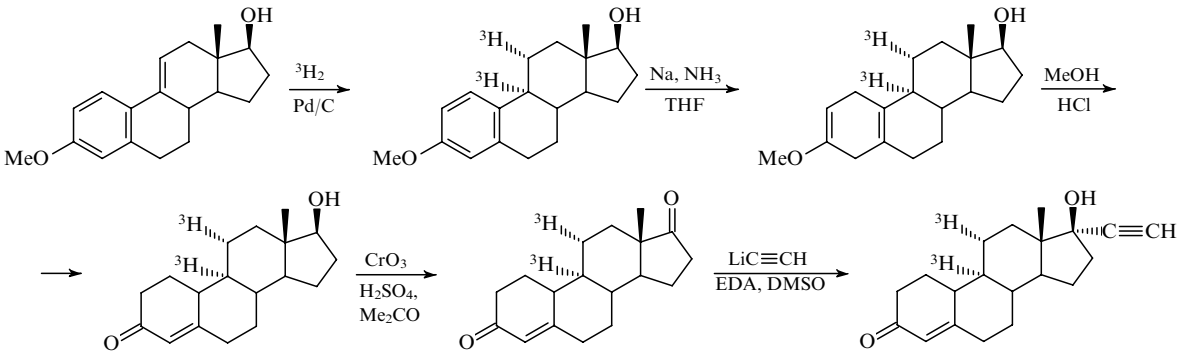
Selective hydrogenation is indispensable for the synthesis of labelled derivatives of natural steroids.[22,129,134] As can be seen from Scheme 19, several tritium-labelled, biologically active progesterone analogues can be prepared from the same precursor (see Table 22).[129]

Table 22. The values of molar radioactivities of labelled steroids.

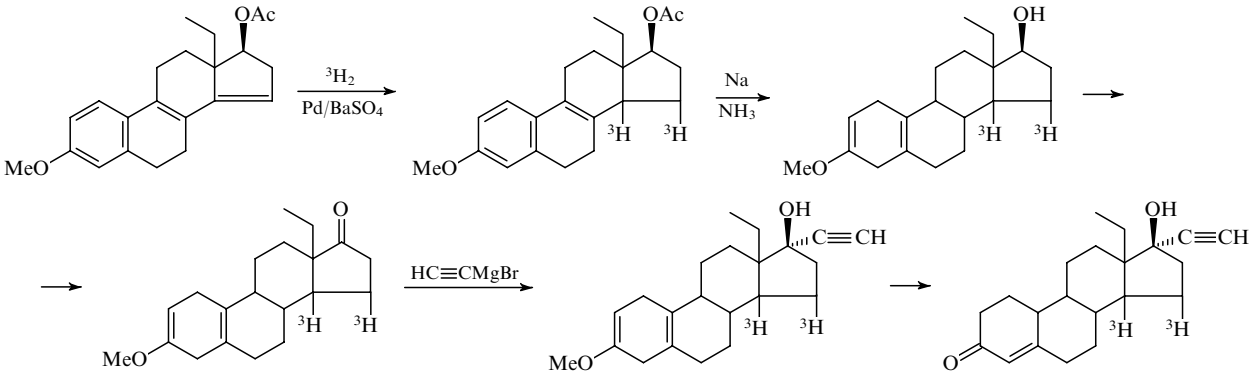
Compound	α/PBq mol^{-1}	Ref.
Liquid-phase isotope exchange		
[G-^{3}H]Cholestan-3β-ol	0.0008	4
β-[G-^{3}H]Sitosterine	0.0021	4
Solid-phase isotope exchange		
5α-[G-^{3}H]Dihydrotestosterone	0.56	120
5α-[G-^{3}H]Dihydrotestosterone enantate	1.50	120
Hydrogenation with gaseous tritium		
17α-Ethynyl-17β-acetoxy-19-nor-[6,7-^{3}H]androst-4-en-3-one oxime	1.6	128
16α,17α[1,2-^{3}H]Cyclopropanopregn-4-ene-3,20-dione	1.5	22
6α-Methyl-16α,17-cyclohexano-[1,2-^{3}H]pregna-1,4-diene-3,20-dione	1.6	22
16α,17α-[3′,4′-^{3}H]Cyclohexanopregn-4-ene-3,20-dione	2.0	22
16α,17α-[3′,4′-^{3}H]Cyclophexanopregna-1,4-diene-3,20-dione	1.5	129
16α,17α-Cyclohex-3′-eno[1,2-^{3}H]pregn-4-ene-3,20-dione	1.6	129
16α,17α-[1,2,3′,4′-^{3}H]Cyclohexanopregn-4-ene-3,20-dione	3.2	129
5α,16α,17α-[1,2,3′,4′-^{3}H]Cyclohexano-pregnane-3,20-dione	3.2	129
5β,16α,17α-[1,2,3′,4′-^{3}H]Cyclohexano-pregnane-3,20-dione	3.2	129
3-*O*-Methyl-[9,11-^{3}H]estradiol	2.1	130
5α-[16,17-^{3}H]Androstan-3-one	1.5–1.7	14
[4,5β-^{3}H]Cholestan-3-one	1.8–1.9	131
17α-Hydroxy-[1,2-^{3}H]androst-4-en-3-one	1.5	132
17α-Hydroxy-5α-[1,2,4,5-^{3}H]androstan-3-one	1.9	132
18-Methyl-3-methoxy-[14α,15α-^{3}H]-estra-1,3,5(10),8-tetraen-17β-ol acetate	2.1	133

The selectivity of the process is achieved through the use of homogenous catalysts. Kinetic studies have shown that hydrogenation of the C(1)=C(2) bond occurs 1.5–2 times more easily than that of the C(3′)=(C4′) bond. However, reduction of the C(3′)=(C4′) bond is still the fast reaction, and after 1–1.5 h the reaction mixture did not contain even more stable 16α,17α[1,2-^{3}H]cyclohex-3′-enopregn-4-ene-3,20-dione. Under optimum conditions (40 min), the reaction mixture contained 10%–15% of 16α,17α-[3′,4′-^{3}H]cyclohexanopregna-1,4-diene-3,20-dione, 20%–25% of 16α,17α-[1,2-^{3}H]cyclohex-3′-enopregn-4-ene-3,20-dione {[^{3}H]CHE} and 60%–70% of 16α,17α-[1,2,3′4′-^{3}H]cyclohexanopregn-4-ene-3,20-dione. Hydrogenation of the latter in the presence of heterogenous catalysts yielded two

Scheme 17



Scheme 18



Scheme 19

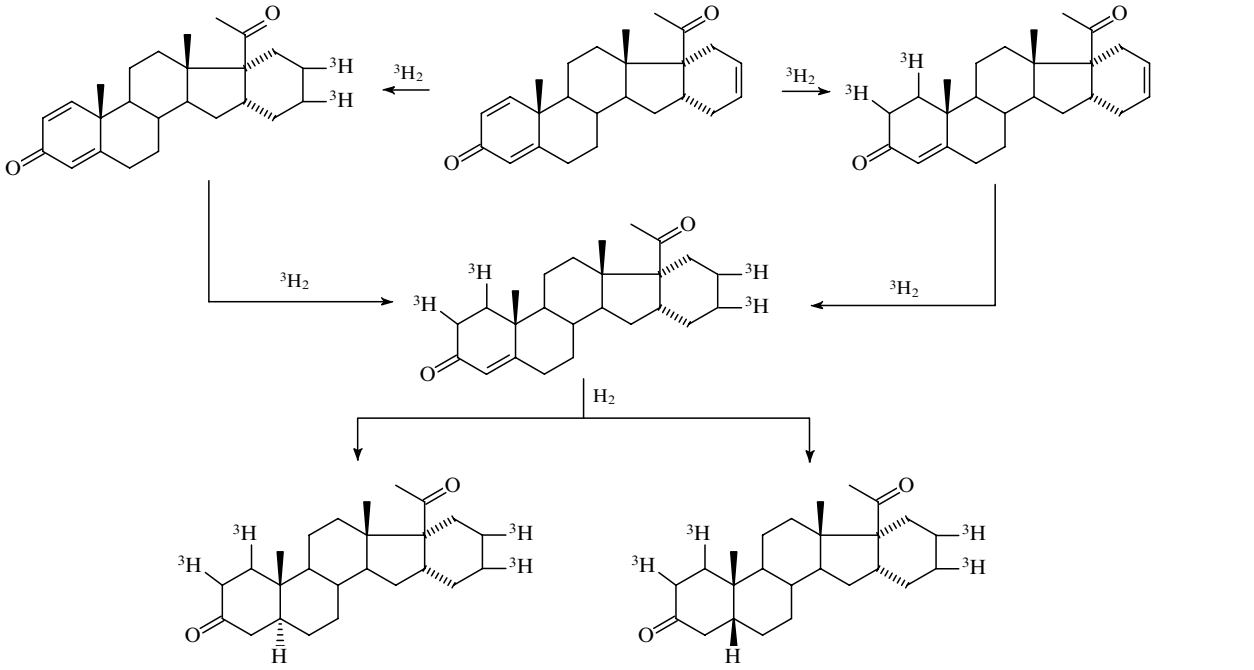


Table 23. The values of molar radioactivities and the yields of labelled steroids obtained by a solid-phase method.[120]

T /°C	τ/h	Target product	α /PBq mol^{-1}
Starting compound — 17-hydroxyandrosta-1,4,6-trien-3-one			
120	5.0	androstanediol	6.65
120	50	androstanol	5.10
150	1.5	androstanediol	5.55
150	1.5	androstanol	4.5
Starting compound — androsta-1,4,6-triene-3,17-dione			
20	1.5	androstenediol	3.10
20	1.5	testosterone	3.14
50	26	androstanedione	4.85
50	26	androstanediol	6.5
50	26	androstanolone	6.5

additional labelled steroids, *viz.*, 5α,16α,17α-[1,2,3,4′-^{3}H]cyclohexanopregnane-3,20-dione and 5β,16α,17α-[1,2,3′,4′-^{3}H]cyclohexanopregnane-3,20-dione.

The use of labelled reagents, *e.g.,* metal borotritides, has been mentioned above (see Table 19). Reactions with these reagents and tritium-labelled methyl iodide and its derivatives afforded labelled steroids, such as [4-^{3}H]- and [16-^{3}H]cholesterols, 1α,3β-dihydroxy-(20*S*)-[4-^{3}H]-(3-hydroxy-3-methylbutyloxy)pregna-5,7-diene, 1α,25-dihydroxy-22-oxavitamin D_3 and some other compounds.[3, 4, 118, 120, 135] The main disadvantage of this method is that the molar radioactivities of the resulting products depend on those of the labelled reagents, which are sometimes too low for conducting biological experiments. Presumably, syntheses of labelled preparations can be based on the use of non-labelled reagents and labelled steroidal precursors and reactions of condensation, oxidation, hydrolysis, acetylation or reduction.[3, 136]

Several labelled 16α,17α-cycloalkano- and 16α,17α-cycloalkeno-substituted progesterones with different sizes of the additional ring *D*′ were synthesised,[22, 129] and their interactions with the progesterone receptor were studied for the first time in direct experiments. The steroid–receptor binding was studied using labelled 16α,17α-cyclohexanopregn-4-ene-3,20-dione {[^{3}H]CHA}, 16α,17α-cyclopropanopregn-4-ene-3,20-dione {[^{3}H]CPA} and proteins from the soluble fraction of rat uterus homogenates responsible for progesterone reception. The interaction of CPA and CHA with the progesterone receptor and other rat uterus proteins was assayed by cross-inhibitory analysis, which is based on the displacement of a labelled ligand from complexes of proteins with non-labelled steroids. The values of relative com-

Table 24. The values of relative competitive activity (RCA) of natural progesterone calculated from the displacement of labelled ligands from complexes with the progesterone receptor from the soluble fraction of pregnant rat uterus.

Labelled ligand	Unlabelled competitor	RCA [a]
[^{3}H]Progesterone	CPA	1.02
	CHA	0.79
[^{3}H]CPA	CPA	0.99
	CHA	0.57
[^{3}H]CHA	CPA	1.57
	CHA	1.00

[a] The progesterone activity was taken as unity.

petitive activity (RCA) were calculated from the concentration ratios of non-labelled compounds inducing 50% inhibition of specific binding of the labelled ligand (Table 24).

It was shown that a progesterone receptor in the soluble fraction of a rat uterus homogenate is a protein that specifically binds CHA and CPA and manifests almost identical parameters of competitive binding. The same regularity was observed with [^{3}H]CHE. This compound binds by the protein which is identical with the progesterone receptor, no other specific binding species in the soluble fraction of rat uterus were detected. This conclusion was confirmed by analysis of CHE inhibitory effect on [^{3}H]progesterone binding. During biological experiments, no metabolic conversions of labelled steroids occur. No significant differences were found in the mode of interaction of [^{3}H]CHE and [^{3}H]progesterone with a receptor isolated from the cells of different animals either. It is interesting to note that CHE as a representative of this class of steroids manifested higher activity in a pregnancy preservation test and a relatively low activity in an endometrium proliferation test upon systemic administration than progesterone.[137] These examples illustrate the possibility of the synthesis of steroid hormones with separated biological functions as regards progestagenic activity.

V. Conclusion

This review describes various methods for introducing tritium labels into biologically active compounds. Recent advances in radiochemistry have made it possible to solve numerous problems concerning the study of processes determining the vital activity of cells and the whole organism. There is no doubt, however, that further progress in medicine and biology will demand elaboration of novel and the development of existing methods of synthesis of labelled compounds. Studies on effects of micromodifications of molecules of various steroids are currently under way. These studies are necessary for the design of a new generation of medicinal drugs possessing strictly specific activity and having no adverse side effects.[138] New approaches to modification of oxylipins and polyenoic fatty acids are being developed in order to elucidate the relationship between the structure and function of these compounds, since the use of natural oxylipins as medicinal drugs is strongly restricted by the wide range of their pharmacological action and rapid metabolism.[106] The synthesis of some derivatives of polyunsaturated acids (*e.g.*, dicarboxylic acids) required for the analysis of enzyme–substrate binding of mammalian 15-lipoxygenases has been carried out. This is important for continuing investigations in the field of gastroenterology, cardiology, angiology, oncology, *etc.*[139,140] A vast variety of compounds (fungicides, immunodepressants, medicinal drugs influencing the central nervous system, pheromones, *etc.*), sometimes having an exotic structure, are being synthesised.[141–143]

Considerable effort is devoted to a search for novel approaches to the synthesis of tritium-containing preparations. These investigations include an elaboration of original procedures for the synthesis of new catalysts and labelled reagents which represent fragments of biologically active compounds or sources of radioactive labels, methods of enzymic synthesis of labelled oxylipins using organic solvents are being developed, *etc.*[144–147] Thus data were obtained showing that enzymes functioning within micelles possess higher stability which increases the efficiency of synthesis of these labelled preparations.[148]

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