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NOTE

THE SYNTHESIS OF TRITIUM-LABELLED ALANINE BY SOLID-STATE CATALYTIC REACTIONS.

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SUMMARY

The solid-state catalytic hydrogenation (SCH) of unsaturated precursor compound and the high-temperature solid-state catalytic isotope exchange (HSCIE) showed that the total incorporation of isotope and its distribution between the α-and β-positions of the alanine molecule can be modified by changing the temperature of solid state reactions. Tritium incorporation at high-temperatures proceeds in a manner which retains the configuration of the asymmetric α-carbon atom. ^{3}H NMR analysis of tritiated isotopomers was used for the investigation of solid-state reactions. The HSCIE reaction proceeds selectively at 140 °C and only part of the molecules is in the the tritium spillover reaction zone. At 220°C HSCIE proceeds evenly along the whole mass to give uniformly labelled alanine.

Key words: Hydrogenation, Isotope exchange, Tritium, Alanine, Isotopomer

INTRODUCTION

The reaction of catalytic hydrogenation of dissolved organic compound and gaseous tritium is most widely used for the preparative synthesis of tritium labelled organic compounds [1]. The tritium label is introduced in saturated compounds by the catalytic isotope exchange reaction of dissolved compound with gaseous tritium (CESG) [2]. The molar activity of the compounds synthesized by this reaction is usually lower than of those obtained by the tritium hydrogenation reaction of unsaturated compounds. The solid-state reaction without solvent provides another approach. SCH and HSCIE proceed in the organic compound mass at elevated temperature in the presence of catalyst metals of the platinum group. For the first time, the HSCIE reaction with gaseous tritium made it possible to synthesize large chemical yields of uniformly tritium labelled optically active amino acids [3]. Tritium incorporation into amino acids synthesized by the SCH is higher, as a rule, than in conventional liquid phase processes. Isotopic exchange of allylic and vynilic hydrogen atoms can take place in the course of hydrogenation [4].

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EXPERIMENTAL

I. The synthesis of L,D-[2,3-^{3}H]alanine by SCH.

2 mg of 2-hydroxyimino-propionic acid, 0.5 ml of water and 20 mg of 6% palladium-on-barium sulphate were placed into a reaction vial. Water was removed by lyophilization. The reaction mixture was evacuated to a pressure of 10^{-3} torr, then tritium was introduced to a pressure of 200 torr. The vial was heated to a temperature of 70-120°C and kept at this temperature during an hour. The vial was then cooled, tritium removed and vial washed with hydrogen.

Tritium-labelled alanine was isolated from the reaction mixture by treating it with 5 ml of 1 M NH_4OH containing 20% of ethanol. The catalyst was filtered off, and the filtrate was evaporated to dryness under reduced pressure. Labile tritium was removed by evaporation under reduced pressure two times for 5 ml with 20% ethanol. Amino acid was isolated by using chromatography on sulphocationic exchanger Amberlite 150-Q in the $^+$H form and ligand exchange chromatography on carboxyl cationic exchanger Amberlite CG 50 (III) in the copper(II) form. 10-20 GBq of L,D [2,3-^{3}H]alanine with specific radioactivity of 1500-2400 TBq/mol was obtained.

II. The synthesis of L-[2,3-^{3}H]alanine by the HSCIE.

Solid mixture comprising platinum group catalyst metal (rhodium, palladium, platinum), 1 mg of L-alanine and 10-100 mg of non-organic carrier (barium sulphate, calcium carbonate, aluminium oxide, carbon), was evacuated to a pressure of 10^{-3} torr, then tritium was introduced into reaction vial to a pressure of 200-400 torr [5]. The HSCIE reaction is carried out at a temperature Of 100-240 °C for 20-200 min. The isolation of amino acid from the reaction mixture is performed as described above for the production of [2,3-^{3}H]alanine by the SCH of 2-hydroxyimino-propionic acid. 2-20 GBq of [2,3-^{3}H]alanine, specific radioactivity 330-3200 TBq/mol, was obtained. Gaseous deuterium instead of gaseous tritium was used in the production of deuterated analogues of alanine.

RESULTS AND DISCUSSION

The introduction of deuterium or tritium in place of hydrogen into organic compounds can lead to considerable isotopic effects. To interpret results of biochemical studies it is useful to know not only isotopic substitution in molecules but also the distribution of the different substitution patterns. Isotopic effects can be used for studying biotransformation mechanisms [6]. Data concerning the isotopomers distribution make it possible to suggest the mechanism of solid-state catalytic reactions that we use for the synthesis of tritium- and deuterium-labelled amino acids.

Table 1 shows results of the gas chromatography/mass spectrometry of bis(trimethylsilyl) derivatives of alanine obtained by HSCIE at 220°C,

containing 2.71 deuterium atoms per molecule on the average. The mass peak 116 m/z was used for the appreciation of the isotopomers distribution Table 1 also shows data calculated for the isotopomers distribution for the mathematical.

Table 1. The isotopomers distribution of deuterium-labelled alanine with the substitution degree equal to 68 % (molar % : I -data of gas chromatography/mass srectrometry; II - model)

	$[^2H_4]$Ala	$[^2H_3]$Ala	$[^2H_2]$Ala	$[^2H_1]$Ala	Ala
I	27	37	22	7	7
II	21	40	28	9	1

model with equiprobable substitution of hydrogen atom for the isotope atom [7]. In the case of alanine obtained by HSCIE at 220°C the distribution of isotopomers is well described by the mathematical model. Isotopomers distribution can be obtained by using ^3H NMR spectroscopy [3]. All 8 possible isotopomers of alanine can be determined in mixture by using proton coupled ^3H NMR. Table 2 shows results of measurements and data calculated for equiprobable isotopic exchange. All tritium isotopomers of the alanine molecule can be presented as $CH_n{}^3H_{(3-n)}CH_m{}^3H_{(1-m)}NH_2CO_2H$ with m=0, n=0,1,2,3 - a,b,c,d, respectively; with m=1, n=0,1,2,3 - e,f,g,h, respectively. Alanine samples of specific radioactivities 2070 and 3200 TBq/mol were synthesized by HSCIE at 220°C. The distribution of isotopomers is well described by equiprobable isotopic exchange. The sample with the specific radioactivity of 330 TBq/mol was synthesized by HSCIE at 140°C. The isotopic label is incorporated selectively at the β-position. Abnormally large quantities of highly substituted forms (e and f) are obtained by HSCIE at this temperature. The quantity of form e in which all the 3 hydrogen atoms at β-position are substituted for tritium is increased by 20-fold as compared with equiprobable model. Hydrogen atoms in the methyl group are chemically equivalent, therefore deviation of isotopomers distribution at HSCIE from equiprobable model is apparently due to the fact that only part of the molecules are accessible to spillover tritium. Data on the content of isotopomers permitted to assess the percentage of a substrate in the reaction zone. Thus, at a temperature of 140°C, only 50% alanine molecules turn out to be inaccessible to spillover tritium. The energy of activated tritium spillover is not sufficient for equiprobable isotopic exchange with hydrogen in both the α-and β-positions of the molecule. There are local areas in the amino

acid layer where the reaction proceeds. Increased production of form e is probably due to the irregularity of HSCIE within the substrate mass at 140°C.

Table 2. The isotopomers distribution (%) of tritium labelled alanine (I -^{3}H and ^{1}H NMR; II - model).

		Specific radioactivity TBq/mol							
		330		2370		2070		3200	
		I	II	I	II	I	II	I	II
Isotopomer	a	0	0.004	13	9	3	5	33	31
	b	0	0.14	16	22	15	17	33	31
	c	0	1.6	8	18	22	18	10	10
	d	1	6.1	4	5	5	6	2	1
	e	1	0.044	16	8	5	6	9	11
	f	4	1.7	21	18	17	19	9	11
	g	21	17	12	15	23	20	3	4
	h	74	72	10	4	7	7	1	0.4

Data from Table 2 show that at 220°C the HSCIE reaction leads to equiprobable isotopic exchange in the whole amino acid mass. The alanine sample with the specific radioactivity of 2370 TBq/mol was synthesized by the SCH at 110°C. A high instance of form completely substituted at the β-position (a and e) is observed with samples synthesized by HSCIE.

Results of isotope label distribution between the α- and β-positions of labelled alanine obtained by SCH (I) and HSCIE (II) are shown in Table 3. It was revealed that in the course of solid state hydrogenation of unsaturated precursor the incorporation of label and the character of its distribution can also change with the reaction temperature. In spite of the fact that the multiple bond is located at the α-carbon atom of the unsaturated compound, the isotopic label preferentially incorporates at the β-position. As the temperature is increased from 70 to 110°C, the increase in specific radioactivity growth is mainly due to tritium incorporation increase at the β-position. The HSCIE reaction makes it possible to obtain both selectively- and uniformly-labelled amino acids. At 140°C the isotopic label preferentially incorporates at the β-position of alanine, while in HSCIE at 220°C the distribution of incorporated tritium corresponds to the stoichiometrical content of hydrogen at the α-and β-positions of alanine. Here hydrogen isotopic exchange at α-position does not lead to complete racemization like in liquid-state

reactions of hydrogen isotopic exchange. The optical purity of [2,3-^{3}H]alanine corresponds to 80%.

Table 3.Tritium distribution in the molecule of alanine with respect to the production method (I - SCH, II - HSCIE).

	Specific radioactivity TBq/mol	Temperature °C	Substitution α	Substitution β	Ratio α / β
I	1590	70	0.27	1.21	4.5
I	2370	110	0.29	1.91	6.5
II	330	140	0.015	0.29	19.3
II	2070	220	0.48	1.45	3.0
II	3200	220	0.75	2.25	3.0

Therefore, solid state processes ensure the production of high-labelled alanine and allow the character of isotope label distribution in the amino acid molecule to be changed over a wide range.

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