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THE SOLID-STATE SYNTHESIS OF TRITIUM LABELLED HETEROCYCLIC BASES

Georgy V.Sidorov, Nikolai F.Myasoedov

Institute of Molecular Genetics, Russian Academy of Science

Moscow, Russia

The results of a study of the solid-state catalytic hydrogenation and the synthesis of tritium labelled native heterocyclic bases are presented. The effect of different palladium catalysts and reaction conditions on yield and molar radioactivity of final compounds was investigated. For some compounds, data on the intramolecular distribution of tritium were obtained by using the isotope exchange reaction and tritium NMR. Tritium labelled purine and pyrimidine bases (25 -180 Ci/mmol.) were synthesized.

Key words: tritium, adenine, guanine, xanthine, hypoxanthine, theophylline, benzyladenine, thymine, uracil, furfuriladenine.

INTRODUCTION

Solid-state catalytic hydrogenation (SCH) was first suggested[1] for the synthesis of tritium labelled nucleic acid components, heterocyclic bases, nucleosides and nucleotides in 1977. In that particular study, one of the possible variants of the reaction, the reductive dehalogenation, was studied. The method proved effective for the reduction of the 5-formyluracil aldehyde group in the synthesis of tritium labelled thymine[2]. The reduction appeared to be accompanied by intense isotope exchange of the formyl group with tritium. We have not hitherto described this process.

In this paper we report on the SCH reaction of purine and pyrimidine bases. The effect of different catalysts and reaction conditions on the yield and molar radioactivity of the compounds produced was investigated. For studying the SCH reaction, a tritium-protium mixture (1:1000) was used. The synthesis of the

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tritium-labelled bases with high molar radioactivity was carried out by using carry-free tritium.

RESULTS AND DISCUSSION

Table 1 shows the results describing the effect of different catalysts on the yield and molar radioactivity (A_{mol}) of purine bases produced in the reaction of SCH with tritium. They show that the largest A_{mol} is achieved with the use of catalyst C (see Experimental) for all the compounds studied.

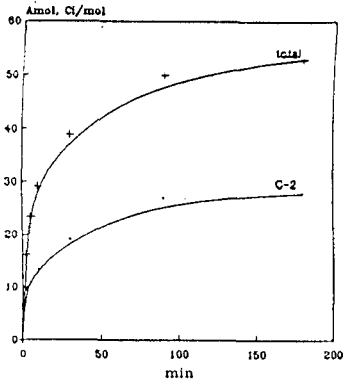
Table 1. The effect of different catalysts on the reaction of SCH of purine bases with tritium at 200 °C.

Catalyst	A_{mol}, Ci/mmol (yield,%)		
	Guanine	Xanthine	Hypoxanthine
A	17.1 (69)	--(traces)	48.4 (83)
B	16.5 (68)	4.6 (72)	19.7 (79)
C	22.4 (75)	25.1 (75)	51.4 (67)

The characteristics of the kinetics curve describing tritium incorporation into adenine (Fig.1) were similar to those reported earlier when the reaction with thymine was studied[3]. Data for curve 2 were obtained after re-exchange of tritium at C-8 adenine (pH 7.0; 100 °C; 16 h). Fig.2 shows the temperature dependence of total tritium incorporation into purine bases. These results indicate that the degree of tritium incorporation increases considerably at temperatures of over 150 °C. Compounds are produced that are practically completely substituted with tritium at stable C-H bonds.

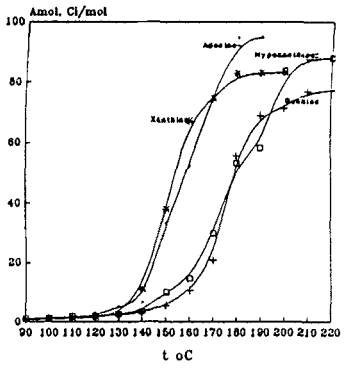
During the study of the SCH reaction of 5-formyluracil with tritium the A_{mol} of the produced thymine appeared to grow rapidly at 140 °C (Fig.3). We attribute this phenomenon to the parallel reaction of isotope exchange with tritium of the 5-aldehyde group uracil hydrogen. This results in thymine practically fully substituted with tritium in the methyl group. It appears that, in C-6 pyrimidine , hydrogen is also involved in the isotope exchange reaction if the temperature is allowed to increase too high and

Figure 1.



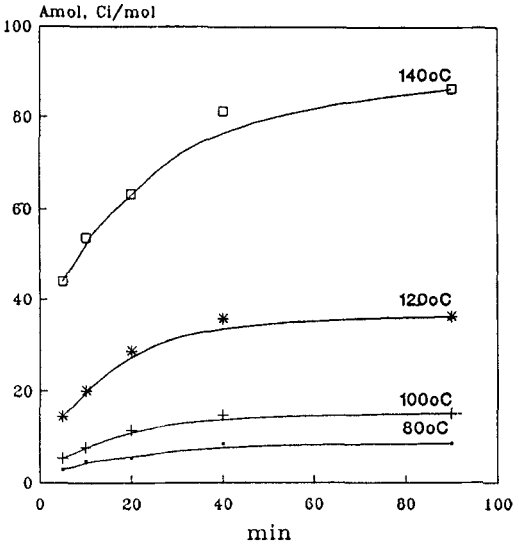
The reaction kinetics of adenine SCH with tritium (1:1000) at 140 °C

Figure 2.



Temperature dependence of isotope substitution data degree in SCH with tritium.

Figure 3.



The reaction kinetics of tritium for 5-Formyluracil

5-formyl, 5-hydroxymethyl and 5-methyluracil are used as precursors. Fig.4 shows the tritium NMR spectra of the thymine obtained in the SCH reaction with tritium of 5-hydroxymethyluracil (a) and thymine (b). These results demonstrate that in both cases tritium-labelled thymine contains tritium in the methyl and C-6 positions at a ratio close to 3:1.

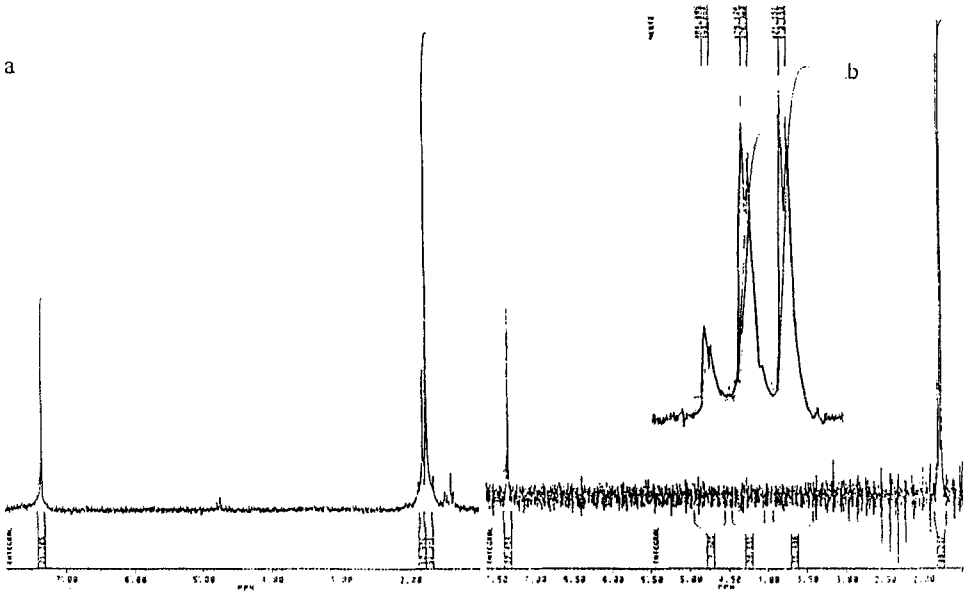


Figure 4. ^{3}H-NMR spectra of thymine produced in the SCH reaction of 5-hydroxymethyluracil (a) and 5-methyluracil (b).

The analysis of the SCH reaction of uracil with tritium shows the yield of tritium-labelled uracil to be strongly dependent on the type of palladium catalyst (Table 2). The yield of labelled uracil decreases with temperature elevation (due to the formation of its hydration product e.g. 5,6-dihydrouracil). The molar radioactivity of uracil does not vary greatly (Table 3).

Table 2. The effect of different catalysts on the reaction of SCH for uracil. Tritium-protium mixture (1:1000).170°C, 5 min.

Catalyst	Yield, %	A_{mol}, Ci/mol
D	24.0	150
E	15.6	108
F	96.0	34.3
G	94.1	57.0

Table 3. The effect of reaction conditions on the SCH reaction for uracil. Tritium-protium mixture (1:1000). Catalyst E.

t °C	Duration, min	Yield, %	A_{mol}, Ci/mol
160	5	32	40
160	10	28	53
160	20	20	80
170	1	60	40
170	3	33	43
180	1	50	42

Table 4 shows some results for the SCH reaction in the synthesis of tritium-labelled heterocyclic bases. Clearly the reaction of SCH with tritium is an important one: it allows tritium-labelled bases contained in nucleic acids to be synthesized from native compounds. The obtained preparations are almost fully tritium-substituted at stable C-H bonds.

Table 4. Tritium-labelled heterocyclic bases obtained in the SCH reaction with tritium.

Compound	t °C	A_{mol}, Ci/mmol	Isotope substitution, %
Adenine	170	54.2	92
Guanine	210	24.9	85
Xanthine	180	25.1	86
Hypoxanthine	200	51.1	88
Thymine [a]	140	85.0	73
Thymine [b]	160	110	94
Thymine [c]	170	112	95
Uracil	170	49	84
Benzyladenine	190	180	88
Theophylline	160	24.8	12
Furfuriladenine	160	160	79

[a] from 5-formyluracil; [b] from 5-hydroxymethyluracil; [c] from 5-methyluracil

EXPERIMENTAL

UV spectra were registered on a SP-16 spectrophotometer. Tritium NMR spectra were registered in D_2O on a AS 250 NMR Bruker spectrometer. The radioactivity of samples was measured on a

liquid scintillation counter using LC-8 scintillation cocktail. The catalysts were obtained from the follows sources: 5% Pd/Al_2O_3 (A) from St.Petersburg; 5% $Pd/BaSO_4$ (B) and 5% $Pd/CaCO_3$ (C) from "Fluka"; different batches of 5% $Pd/BaSO_4$ (D,E,F) and 10% $Pd/BaSO_4$ (G) were prepared according to the literature procedure[4]. Tritium-labelled heterocyclic bases were chromatographically isolated on a Sephadex column (16 x 900 mm), eluent - water 25 ml/h. HPLC was performed on a 4.6 x 250 mm. column with Nucleosil 120-5 C18, mobile phase - 2% acetonytrile in 0.1 mol/l triethylammoniumbicarbonate, pH 7.0. The rate of flood was 1.0 ml/min, registration at 254 nm.

The SCH reaction. Solid mixture of the initial compound and catalyst was placed in a glass vial. Upon evacuation, gaseous tritium (95-97%) or tritium-protium mixture (1:1000) was introduced. Then the vial was thermostated at a chosen temperature for a specified time, then cooled and the excess tritium removed. The reaction products were washed with water, and the catalyst separated by filtration. Labile tritium was removed by evaporation to dryness at 37°C. The dry remainder was dissolved in water, and the isolation of the labelled compounds was performed as indicated.

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