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Research Article

Synthesis of tritium labelled zaleplon

V.P. Shevchenko[1,*], I.Yu. Nagaev[1], N.F. Myasoedov[1] and A. Susan[2]
[1] *Institute of Molecular Genetics, Russian Academy of Sciences, Kurchatov sq., 123182 Moscow, Russia*
[2] *International Isotopes Clearing House, Inc., 9110 Lee blv./P.O. Box 6986, Leawood, KS66206, USA*

Summary

The reaction conditions for the incorporation of tritium into zaleplon have been investigated. The methods studied were the catalytic isotope exchange reaction with tritium gas and catalytic isotope exchange reaction with tritiated water. The results showed that the latter method was the method of choice giving a compound with a higher specific activity and a better yield. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: tritium; zaleplon; catalytic isotopic exchange

Introduction

To study processes occurring in cells *in vivo*, tritium labelled preparations are usually needed.

Zaleplon is a promissing benzodiazepine receptor agonist with potent sedative and hypnotic therapeutical action. The detailed investigation of its biological activity needs a corresponding labelled analog. The purpose of our work was to synthesize [^{3}H]zaleplon (**I**) with a specific activity higher than 10 Ci/mmol.

*Correspondence to: V.P. Shevchenko, Institute of Molecular Genetics, Russian Academy of Sciences, Kurchatov sq., 123182 Moscow, Russia. E-mail: nagaev@img.ras.ru

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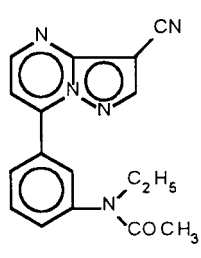
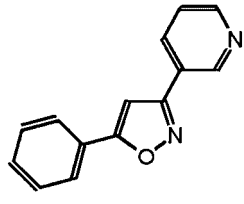
Tritium labelling of heterocyclic compounds has been widely studied.[1–3] It has been shown that various types of aromatic rings differ greatly in their stability and degree of tritium incorporation, as shown by the labelling of 3-pyridine-5-phenyl-isooxazole (**II**), ribavirine (**III**), tiazofurine (**IV**), alprozalam (**V**) via a method often used for such compounds, i.e. the solid-phase catalytic change (see method 1). The yields of the resulting tritium labelled compounds ranged from 5 up to 80% and their specific radioactivity, from 0.6 to 30 Ci/mmol (Table 1).



(I)



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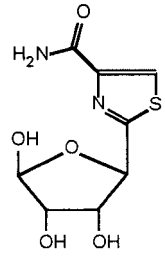

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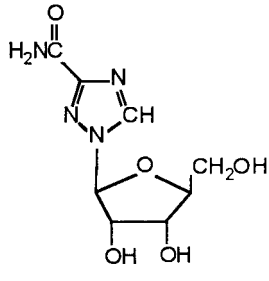

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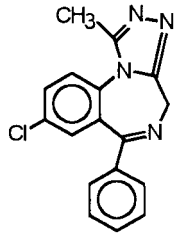

(V)

Experimental

The optimum conditions for the high temperature catalytic isotope exchange labelling of zaleplon were determined by studying the dependence of the specific activity and the yield upon the reaction time (10–90 min), the nature of the catalyst (PdO, 5% PdO/Al_2O_3, 5%Pd/$BaSO_4$, 5%Pd/$CaCO_3$, 5%Pd/C), the catalyst-to-substrate ratio (3:1–10:1), and the reaction temperature (60–220°C).[5–7] The analysis and purification was achieved by thin-layer chromatography (TLC) or higher performance liquid chromatography (HPLC) techniques (Table 2).

Table 1. Yield and specific radioactivity of tritium labelled compounds (I)–(V)

Substance	(I)	(II)	(III)	(IV)	(V)
Specific radioactivity (Ci/mmol)	4–5	0.6–0.7	27–30	17–18	3–4
Yield (%)	2–3	20–25	70–80	45–50	5–10

Table 2. Retention time and R_f values for zaleplon

TLC on Silufol silica plates		
System	*Description*	R_f
A	methylene chloride – methanol (95:5)	0.79
B	ethylacetate – hexane – acetic acid (75:25:1)	0.63

HPLC		
System	*Description*	*Retention time (min)*
C	Lichrosphere C_{18}, 5 µm (2 × 60 mm), 0.1 ml/min, acetonitrile – 25 mM ammonium dihydrophosphate, pH 3 (1:1)	2.57
D	Lichrosphere C_{18}, 5 µm (2 × 60 mm), 0.1 ml/min, acetonitrile – 25 mM ammonium dihydrophosphate, pH 3 (2:3)	4.17
E	Silasorb C_{18}, 13 µm, 10 × 250 mm, 2 ml/min, acetonitrile – 25 mM ammonium dihydrophosphate, pH 3 (45:65)	13.68

Synthesis of [^{3}H]zaleplon ([^{3}H]I) via isotopic exchange with tritium gas

Zaleplon (3 mg) was coated onto 30 mg of 5% Pd/$BaSO_4$ and the resulting mixture placed in the reaction vessel. The latter was evacuated to a pressure of 0.1 Pa and tritium gas was introduced to a pressure of 40 kPa. The reaction was carried out by heating this mixture for 5 min at 180°C. The reaction products were extracted from the catalyst with methanol (5 × 1 ml) and the labile tritium was removed by repeated evaporation of the solvent. The resulting crude product was purified by preparative TLC using system (B): the desired compound was extracted with methanol (3 × 10 ml), filtered, evaporated and subjected to HPLC purification using system (E). The desired pure product was isolated in 2–3% yield and had a specific radioactivity of 4.6 Ci/mmol.

Synthesis of [^{3}H]zaleplon ([^{3}H]I) by catalytic isotopic exchange with tritiated water generated in situ

A mixture of 30 mg of reduced 5% PdO/Al_2O_3, ^3H_2O (obtained from 20 mg PdO as described in Reference 8). 30 µl of dioxane, and 10 mg of zaleplon were placed in a gas reaction ampoule which was then sealed and heated at 180°C for 40 min. The reaction mixture was treated as described above. [^{3}H]zaleplon with a specific radioactivity of 18.5 Ci/mmol and yield of 40–45% was obtained.

Results and discussion

The results of our experiments showed that the values of the specific activity of [^{3}H]zaleplon ranged from 0.78 to 4.6 Ci/mmol depending on the reaction conditions while at temperatures above 180°C complete hydrogenation of zaleplon occurred (Table 3). These results lead to the conclusion that the high temperature solid-phase catalytic exchange reaction was not a desirable method for tritium labelling of zaleplon.

Table 3. Influence of catalyst nature and reaction conditions on specific radioactivity of zaleplon (catalyst to substrate ratio equals 10:1)

Time (min)	Temperature (°C)	Specific rad. (Ci/mmol)
	5% PdO/Al_2O_3	
5	140	1.8
15	140	2.3
5	160	2.1
15	160	2.6
5	180	2.4
15	180	3.9
	5% Pd/C	
5	180	1.2
15	180	1.5
	5% $Pd/CaCO_3$	
5	180	0.8
15	180	1.2
	5% $Pd/BaSO_4$	
5	180	3.9
15	180	4.6
5	200	Completely hydrogenated
5	220	Completely hydrogenated

A more complex method – the isotope exchange with tritiated water at temperatures above 100°C[3] – was then tried. The technique consisted of the *in situ* synthesis of tritiated water of high specific activity via reduction of PdO in an atmosphere of gaseous tritium, at temperatures between 70 and 80°C, in the presence of the substrate to be labelled. The resulting mixture of tritiated water, activated catalyst and solution of the substrate were then sealed in an ampoule and heated for 30–120 min at temperatures between 100 and 200°C. Under the optimum conditions, a specific activity of 18.5 Ci/mmol and a yield of 45% was obtained (see method 2). Thus this appeared to be the more successful approach.

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