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SYNTHESIS OF PHYSIOLOGICALLY ACTIVE TRITIATED COMPOUNDS USING HIGH SPECIFIC ACTIVITY TRITIATED WATER

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SUMMARY

A number of physiologically active compounds - steroid hormones, some phytohormones, carbohydrates and nicotinamide adenine dinucleotide - have been tritiated using high specific activity tritiated water produced by the oxidation of tritium gas on palladium oxide and in the presence of a dry organic solvent. In the process the effect of various variables such as catalyst and solvent have been investigated. The products so obtained had specific activities in the range 3 - 25 Ci/mmol.

Key Words: heterogeneous and homogeneous catalysts, tritium gas, tritiated water, phytohormones, steroids, carbohydrates, nicotinamide adenine dinucleotide.

Hydrogen isotope exchange reactions with tritium-containing solvent are widely used for the synthesis of tritium labelled compounds. More often than not,

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such reactions are performed in a high alkaline water solution [1]. Tritiated water is the most commonly employed tritiated solvent. Acids and bases, both homogeneous and heterogeneous catalysts [2], and isotope exchange at elevated temperatures [3,4] are amongst the variables used. The molar radioactivity (A_{mol}) of the compounds produced via these procedures is limited by the specific activity of the tritiated water. Usually its value does not exceed 100 - 200 Ci/ml (3 - 6% of the maximum attainable value, for example, 40 Ci/ml in [3]). Tritiated water with a higher specific activity is unstable due to autoradiolysis. We have previously proposed that the tritiated water can be dissolved in an inert organic solvent [5]. In this case, the activity of the solution is determined by the amount of tritiated water present. Tritiated water with a very high specific activity can be produced, for instance, by oxidising tritium gas in a dry organic solvent.

The purpose of the present work was to study the hydrogen isotope exchange of a number of physiologically active compounds: steroid hormones, some phytohormones, carbohydrates and nicotinamide adenine nucleotide with highly active tritiated water. The latter was produced by oxidising tritium gas (95 - 97 %) on palladium oxide in a dry organic solvent (contents of water less than 0.05%). We studied the effect of different heterogeneous and homogeneous catalysts, and the concentration tritiated water on the yield and specific activity of the resulting compounds.

RESULTS AND DISCUSSION

Table 1 contains some data, describing the catalytic heterogeneous isotopic exchange reaction of 17α-methylandrostandiene-1,4-ol (MN) with gaseous tritium.

These results show that the steroid is rather easily hydrated. No isotope exchange was observed in those cases where the hydration reaction could be totally inhibited (by adding pyridine or using Lindlar's catalyst). Similar results were obtained for 17α-methylandrostandiene-5-diol-3β-17α (ML). Therefore,

Table 1. Some results of the catalytic heterogeneous isotope exchange reaction of MN with gaseous tritium (0.01 %).

Catalyst	Solvent	Some experimental results
5% $Pd/BaSO_4$	Ethanol	Hydration
5% $Pd/BaSO_4$	Ethanol-pyridine	Practically no isotope exchange
5% Ru/Al_2O_3	Ethanol	Practically no isotope exchange
5% Rh/Al_2O_3	Ethanol	Rapid hydration
5% Pt/asbestos	Ethanol	Slow hydration
Lindlar's	Ethanol	Practically no isotope exchange

further research was devoted to studying the hydrogen isotope exchange reaction with tritiated water. The reaction of progesterone with tritiated water at temperatures between 20 - 100 °C (20 hours) without a catalyst was not effective - the maximum molar radioactivity of progesterone did not exceed 5.4 mCi/mmol. The maximum specific activity of the labelled steroids (progesterone, estriol, and cholesterol) was obtained when the concentration of tritiated water in the organic solvent was 2% or greater (Fig. 1). Increasing the temperature from 20 to 100 °C resulted in insignificant (about 30%) increase in the specific activity although the yield of by-products markedly increased.

The influence of various catalysts on the isotopic exchange with tritiated water is summarised in tables 2 – 4. These show the best results are reached, as a rule, with 5% Pd/Al_2O_3. For ribose and 2'-desoxyribose the maximum specific activity was achieved with a PdO catalyst.

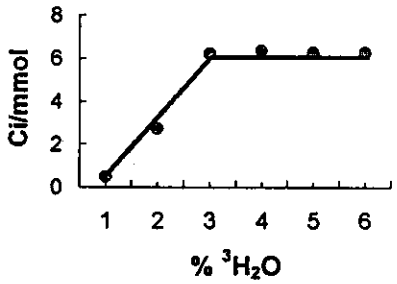

Figure 1. Relationship between the concentration of tritiated water and the specific activity of some steroids.

The isotope exchange of 6-benzylaminopurine in 100 µl DMPA (Tab. 2), ribose and 2'-deoxyribose in 100 µl dioxan (Tab. 3) with tritiated water (15 µl; 2.0 Ci/ml), 10 hours. Temperature 20 °C.

Table 2.

Catalyst (mg)	A_{mol} (% of A_{mol} of tritiated water)
A (20)	9.0
F (30)	35
G (10)	2.6
H (10)	11
I (10)	8.2
J (10)	9.5
Pyridine (10 µl)	6.6

Table 3.

Catalyst (mg)	pH	A_{mol} (% of A_{mol} of tritiated water)
F (50)	7	5 - 6
F (50)	12	3 - 4
F (50)	3	3 - 4
H (10)	7	20 - 22
H (10)	12	11 - 13
H (10)	3	22 - 24

The specific activity of the labelled compounds depends on many factors (temperature, duration of reaction, specific radioactivity of tritiated water, the efficiency of the catalyst, the nature of the solvent and of the studied compound). The increase in temperature, specific radioactivity of tritiated water and duration of reaction increases the degree of decomposition of the labelled compounds.

Table 4. The catalytic isotope exchange of IAA and BAP with tritiated water (5 μl) in dioxane (95 μl), 10 hours. Temperature 20 °C.

Compound	Catalyst	A_{mol} (% of A_{mol} of tritiated water	The note
BAP	F	23	
BAP	H	1.6	
BAP	F	27.5	1% TEA
BAP	F	31	5% TEA
BAP	F	17	10% TEA
BAP	-	1.8	
IAA	H	7.6	5% TEA
IAA	F	8.5	
IAA	A	6.0	

Therefore, determination of the optimum duration of reaction will lead to the optimum yield of labelled compounds and the highest specific activity. From the data, listed in Table 5, the optimum duration of reaction is in the region of 10 hour.

Table 5. Relationship between duration of reaction and yield and molar activity of steroids.

Dur., h.	Progesterone		Estriol		Cholesterol	
	Yield, %	Ci/mmol	Yield, %	Ci/mmol	Yield, %	Ci/mmol
2	95	3.10	95	2.30	95	0.48
4	90	4.86	85	5.05	85	1.22
6	85	6.29	75	8.48	80	1.48
10	70	6.48	60	10.5	65	1.76
24	20	6.35	18	10.0	15	1.68
50	10	6.48	10	10.1	10	1.65

Table 6. Tritium labelled compounds produced in the hydrogen isotope exchange reaction with highly active tritiated water.

Compound	Ci/mmol	Compound	Ci/mmol
Progesteron	6.70	Zeatin	18.0
Estradiol	10.8	Zeatinriboside	16.5
Cholesterol	5.9	Indolylacetic acid	9.8
Cortisol	5.9	Benzyladenine	24.5
17α-methylandrostandiene-1,4-ol	5.4	Fusicoccin H	10.8
17α-methylandrostandiene-5-diol-3β-17α	6.1	Nicotinamide adenine dinucleotide	4.5
Gibberellin A_1	5.5	Ribose	7.1
Gibberellin A_3	4.9	2'-Deoxyribose	6.5

Table 6 lists the tritium labelled compounds obtained in the hydrogen isotope exchange with tritiated water. The results show that the specific activity of the produced compounds to be high. The tritium is incorporated both in compounds of rather simple structures (ribose, 2'-deoxyribose) and in more complex compounds, such as gibberellins, fusicoccine-H and nicotinamide adenine dinucleotide. The method clearly allows one to reach high specific activities for compounds containing easily reduced or hydrated groups, such as gibberellins, 17α-methylandrostendiene-1,4-ol, 17α-methylandrostendiene-5-diol-3β-17α, zeatin and zeatinriboside. For these compounds, it is very difficult, and sometimes impossible, to perform catalytic reactions with gaseous tritium.

EXPERIMENTAL

The choice of the reaction conditions for the hydrogen isotope exchange reaction was determined using tritiated water with a specific activity of 2.0 Ci/ml (38 Ci/mol). The subsequent synthesis of the labelled compounds was carried out

using highly active tritiated water produced by oxidation of gaseous (with concentration 95 - 97%) tritium. The dry solvents were obtained according to standard procedures and stored above a 4A molecular sieve.

The following catalysts were employed: 5% $Pd/BaSO_4$ (A), 5% Ru/Al_2O_3 (B), 5% Rh/Al_2O_3 (C), Pt/asbestos (D), - «Fluka»; 5% $Pd/BaSO_4$ - Lindlar's catalyst (E), 5% Pd/Al_2O_3 (F), PtO_2 (G), PdO (H), bis(triphenylphosphin)-palladium(II)-chloride (J) - «Merck».

Hydrogen isotope exchange with tritiated water. The compound, dissolved in 95 μl of a dry organic solvent (methanol, dioxane, ethyl acetate, DMPA), the catalyst and 5 μl of tritiated water were placed in a glass vial. The isotope exchange reaction was performed with stirring of the reaction mixture. After the reaction was completed, the solvent was isolated by distillation in vacuum. The catalyst was separated by centrifugation. Labile tritium was removed by evaporation with 10 ml of 50% ethanol, and this operation was repeated two times.

Hydrogen isotope exchange using highly active tritiated water. 100 μl of a dry organic solvent and 21 mg of PdO were placed into a reaction ampoule, which was connected to a tritium gas line. The ampoule was then frozen in liquid nitrogen, evacuated and filled with gaseous tritium (up to pressure of 300 mm Hg). After defrosting, the reaction mixture was stirred at room temperature. After removal of gas, the resulting solution of tritiated water was removed in vacuum into another reaction ampoule, containing the catalyst and the compound to be labelled. The resulting solution was stirred at room temperature. The separation of the solvent and the removal of labile tritium was conducted as described above.

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