Synthesis of [β-4,5-^{3}H]-Cholestan-3-one, a Trail Following Pheromone Component of the Eastern Tent Caterpillar

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Summary

Hydrogenation of 4-cholesten-3-one (**1**) with tritium gas over palladium black in pyridine afforded [β-4,5-^{3}H]-cholestan-3-one (**2a**). Tritium NMR showed that there was a mixture of tritiated compounds formed, **2a-2c**; the latter two containing small amounts of protium substitution. Tritium NMR also indicated that none of the unwanted α-diastereomer was formed.

Keywords: 5β-cholestan-3-one, eastern tent caterpillar, *Malacosoma americanum*, pheromone, tritium labeled.

Introduction

A trail pheromone component of the eastern tent caterpillar has been identified as 5β-cholestan-3-one (*Malacosoma americanum* F.).[1] Foraging larvae (caterpillars) mark trails in search of food; successful foragers over-mark these trails when returning to the nest. It has been shown that these trails lose biological activity after some period of time.[2] To study the mechanism by which activity is lost, we intend to conduct experiments at concentrations close to biological conditions (approximately 10 pg/mL), concentrations well below the detection limits of modern GC, GCMS, and HPLC methods.

Results and Discussion

Introduction of tritium into 4-cholesten-3-one (**1**) is achieved by reaction of carrier free tritium gas over palladium black (10% Pd-OH/C). Catalytic hydrogenation of 3-oxo-4-ene steroids using palladium catalysts is a convenient route to 5β-steroids.[3] The stereoselectivity which provides the 5β-configuration depends on the reaction medium and the functional groups associated with the steroid[4]. It has been

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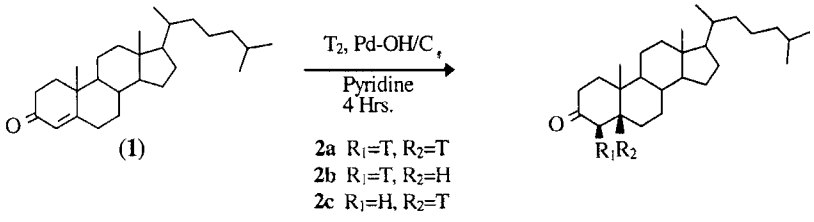
shown that catalytic hydrogenation of 4-cholesten-3-one can be achieved with stereoselectivity in favor of the β-configuration ranging from 93.9 to 99.5 percent diastereomer excess (% d.e.) in various solvents.[3]



Scheme 1: Synthesis of [β-4,5-^{3}H]-cholestan-3-one.

Tritium NMR (Table 1) was used to determine the purity of the radiolabled compound by determining the % d.e., and the extent of exchange of either a proton or a tritium at other positions of the molecule. For our purposes (scintillation counting), the location of the tritium substitution was not as crucial as the purity at the 5β-position.

position	chemical shift (ppm)	tritium coupling (Hz)	proton coupling (Hz) vicinal	geminal
4β-tritium	1.9	5.4	3.6	16.0
5β-tritium	1.7	5.4	3.6-5.5	14.0

Table 1: 300 MHz [^{3}H] NMR spectrum of [β-4,5-^{3}H]-cholestan-3-one.

From the proton decoupled spectrum, we confirmed that none of the unwanted 5α-epimer formed. Analysis of the product by TLC showed no diastereomeric spots, and tritium NMR showed no extra diastereomeric peaks. Also, we determined that less than 3% of the hydrogens at the 4α-position had exchanged with tritium; this is evident by the presence of two small peaks at δ 2.63. A combination of proton-decoupled and proton-coupled spectra suggest that we produced a mixture of 3 products in our reaction (**2a:2b:2c**) in a ratio of 2:1:1 (Scheme 1). Compound **2a** has two tritium atoms, one at the 4β-position and one at the 5β-position; compounds **2b** and **2c** have a single tritium addition at positions 4β and 5β-respectively.

Experimental

Commercial grade reagents were used without further purification and the pyridine used was dried over 4Å molecular sieves. Tritium NMR spectra were recorded on a Bruker AMX 300 MHz instrument at the tritium frequency of 320.135 MHz in deuterated chloroform. Scintillation counting was performed on a Packard 1500 Tri-carb Liquid Scintillation Analyzer.

[β-4,5-^{3}H]-cholestan-3-one (2a): 9 mg of 4-cholesten-3-one (0.0234 mmol) was dissolved in 1 mL of dry pyridine in a 5 mL flask. The flask was attached to the tritium manifold and 4 mg of the palladium catalyst was placed in a glass spoon above the flask. The flask was then frozen in a liquid nitrogen bath, the pressure reduced, and flushed with nitrogen to remove all traces of water and oxygen (repeated 4 times). The reaction flask was cooled to 78° K and tritium gas was vented into the flask at atmospheric pressure. The solution was allowed to warm to room temperature and the spoon holding the catalyst was inverted introducing it into the reaction mixture. This mixture was allowed to stir for 4 hours at room temperature after which the reaction was flushed with nitrogen to remove excess tritium gas. The solution was cooled to 78° K and the pressure was reduced to degas the solution of all remaining tritium gas (repeated 3 times). The volume of the solution was reduced to half by "pumping off" 0.5 mL of pyridine. This solution was treated with 1 mL of 10% HCl (aq.) and extracted with 5 mL of hexane which was lyophilized overnight. The product was taken up in a minimum amount of a 49:1 hexane:ethyl acetate solution (0.25mL) and passed through a short column of silica gel to separate product from any remaining starting material. The solvent was removed to afford 0.7 Ci of purified product (54% yield) at a specific activity of 51.5 Ci/mmole.

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