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Tritiation and characterization of several suicide substrate enzyme inactivators

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HIGHLIGHTS

- This paper describes the synthesis of [^{3}H] pargyline and [^{3}H] caracemide.
- Several diverse tritiation methods were used to accomplish these labellings and include halogenation–tritium dehalogenation as well as the preparation of [^{3}H] methyl isocyanate.
- This paper also describes the characterization of these substances by tritium NMR.

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ABSTRACT

Methods are presented to tritiate the enzyme inhibitors pargyline and caracemide.

1. Introduction

It has been over 180 years since the discovery of the first enzyme, diastase, by Anselme Payen and since that time, several thousand enzymes and their specific biochemical reactions have been discovered. No doubt, even more enzymes and their critical functions are yet to be revealed. The study of enzyme inactivation is now of intense interest with its likelihood of providing both valuable insight into detailed enzymatic mechanisms as well as identifying new targets for therapeutic intervention. A recent example of this effort is the development of gamma vinyl GABA, an irreversible substrate for the enzyme GABA transaminase, with potential use for various CNS disease indications. Because of our ongoing interest in radiolabelling irreversible enzyme inactivators (Ahern et al., 2003) and in order to provide more useful biochemical tools for the study of enzyme systems, we endeavoured to tritiate two irreversible enzyme inactivators of diverse structure and function.

2. Experimental

Evaporations were carried out on a Buchi rotary evaporator (Model RE 111) at bath temperatures less than 40 °C. Analytical and preparative thin layer chromatography (TLC) were accomplished on Analtech plates coated with silica gel. Autoradiography was done at 0 °C after spraying with 2,5-diphenyloxazole and exposing the plates to X-ray film. TLC plates were also scanned for radioactivity (~370 kBq) using a Vanguard Autoscanner. Analytical high pressure liquid chromatography (HPLC) was performed on a PerkinElmer instrument with peak detection done simultaneously by UV (280 nm) and a IN/US Systems Beta RAM Model 3 radioactivity detector. Solution radioassays were conducted with a PerkinElmer Tri-Carb 3100 T instrument. The NMR spectra were recorded on a Bruker 300 MHz instrument with chemical shifts being reported as parts per million (ppm) downfield from internal trimethylsilane. All chemicals used were reagent grade.

2.1. [^{3}H] pargyline (**2**) from precursor **3**

To a solution of 20 mg (0.1 mmol) of precursor **3** (Aldrich catalogue item 631159) in 3 ml of dry triethylamine with 20 mg of 10% palladium on charcoal was introduced 2.22 TBq of tritium

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gas and the reaction was stirred for 15 min at ambient temperature. After this time, four drops of 2 M hydrochloric acid were added and labile tritium was removed by several vacuum evaporations of ethanol and the reaction was filtered free of catalyst, giving 363 GBq of crude product. This ethanol solution was reduced in volume to 0.5 ml by rotary evaporation and to it were added 3 ml of THF, 0.2 ml of triethylamine and 0.03 ml (0.34 mmol) of propargyl bromide. The reaction was stirred overnight under nitrogen at ambient temperature. The reaction mixture was then evaporated to a small volume and purified by preparative TLC on four 1000 μm silica gel plates eluted with hexane:diethyl ether:ammonium hydroxide (70:30:1). After development, each plate was air dried and visualized by UV. A major band was scraped away from each plate, combined together and eluted with three 20 ml portions of ethanol to afford 35 GBq (a 47% yield based on precursor **3**) of product **2** which was >98% radiochemically pure and co-chromatographed with authentic **1** (Aldrich catalogue item P8013) on both HPLC (microphenyl column eluted with water:THF:diethylamine (65:35:0.2)) as well as TLC (silica gel developed with hexane:diethyl ether:ammonium hydroxide (70:30:1)). The specific activity of product **2** was measured to be 0.75 TBq/mmol by radioassay with UV (ethanol) spectroscopy where $E_{264}=258$ for **1**. The distinctive UV (ethanol) spectrum of **2** was also superimposable on that of **1**. A proton decoupled tritium NMR (CD_3OD) of **2** prepared by this method is shown in Fig. 1.

2.2. [N-methyl-^{3}H] caracemide (**6**)

To a solution of 375 mg (2.5 mmol) of silver cyanate in 2 ml of 1, 2-dimethoxyethane was introduced 139 GBq of tritiated methyl iodide (2.5 mmol) by vacuum transfer and the reaction was stirred overnight at ambient temperature in the dark. After this time, the reaction mixture was vacuum transferred to a second breakseal glass flask containing 188 mg (2.5 mmol) of acetohydroxamic acid (**8**, Aldrich catalogue item 159034) and the reaction was stirred overnight at ambient temperature. After this time, labile tritium was removed by several vacuum evaporations of methanol and the residue was dissolved in 3 ml of chloroform. A radioassay of the solution revealed 2.89 GBq of total activity. Methyl isocyanate (0.050 ml, 0.8 mmol) was then added to the reaction and it was stirred overnight at ambient temperature. After this time, analytical TLC (silica gel eluted with chloroform:methanol (6:1)) indicated an essentially complete conversion of intermediate **9** to product **6**. The crude product was purified by flash chromatography on silica gel eluted with chloroform:methanol (9:1). Column fractions were monitored for activity and combined to afford 1.34 GBq (a 10.3% yield based on silver cyanate) of product **6** which was >98% radiochemically pure and co-chromatographed with authentic **5** on reverse phase HPLC (eluted with acetonitrile: water (92:8)). The specific activity of product **6** was measured to be 5.19 GBq/mmol by gravimetric radioassay. A proton decoupled tritium NMR ($CDCl_3$) of **6** prepared by this method is shown in Fig. 2.

3. Results and discussion

Monoamine oxidase (MAO) is a mitochondrial enzyme which oxidatively demethylates many important neurotransmitters and occurs in two isoforms termed MAO-A and MAO-B. Pargyline (**1**) is an irreversible enzyme inhibitor that is selective for the MAO-B isoform (Ucar, 2002). In choosing a radiolabelling strategy for pargyline, the methylation of a desmethyl precursor with [^{3}H] methyl iodide was initially considered. However, concern that the possible metabolic demethylation of [methyl-^{3}H] pargyline (Weli and Lindeke, 1986) would limit its utility for some biological applications prompted us to turn to another tritiation method.

The phenyl ring of pargyline emerged as an attractive alternative site for tritium installation. Although we have effectively utilized direct catalytic tritium exchange of aromatic rings before (Filer, 2010), our practical experience has been that the sequence of aromatic ring halogenation–catalytic tritium dehalogenation is far milder and routinely provides higher specific activity tritiated products (Egan and Filer, 2013). Ideally, either a brominated or iodinated precursor is desirable for this tritiation approach (Filer, 2013) and the commercial availability of bromo intermediate **3** made it an attractive choice for the precursor. The presence of the reduction vulnerable alkyne group of pargyline compelled us to introduce tritium prior to propargyl group installation. For that reason, bromo precursor **3** was directly tritiated to intermediate **4**. Since the benzyl moiety of precursor **3** could also undergo facile catalytic hydrogenolysis (Cheng et al., 2009), we needed to carefully select tritiation conditions to avoid or at least minimize this side reaction.

Former Pharmacia & Upjohn radiochemists faced a very parallel situation for the catalytic tritium debromination labelling of fluoxetine (Hsi and Stolle, 1996) and found that the use of pyridine as solvent and minimal tritiation reaction time suppressed their debenzylation side reaction. We employed similar conditions for the tritiation of **3** as shown in Scheme 1 and successfully prepared intermediate **4**. However, instead of purifying **4**, we

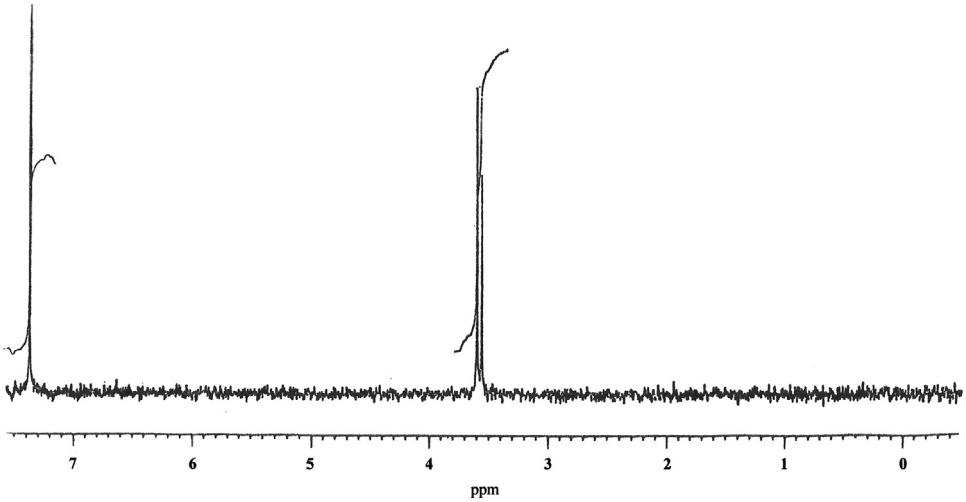

Fig. 1. Proton decoupled tritium NMR (CD_3OD) of **2**.