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Research Article

Synthesis of deramciclane* labeled with tritium in various positions

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Summary

[(1*R*)-*endo*]-(+)-3-bromocamphor was dehalogenated with tritium gas to [3-^{3}H]camphor and via [3-^{3}H]phenylborneol converted to [3-^{3}H]deramciclane isolated as the fumarate salt (specific activity 51.8 GBq/mmol). This three step synthesis from [3-^{3}H]camphor gave an overall yield of 22%.

Benzyloxy-acetic acid methyl ester was reduced with sodium-borotritide to 2-benzyloxy-ethanol-[1-^{3}H], and through a four step procedure was converted to 2-dimethylaminoethyl-[2-^{3}H] chloride. The latter was condensed with the sodium derivative of 2-phenylborneol giving rise to [2-dimethylamino-[2-^{3}H]ethoxy]deramciclane isolated as the fumarate (specific activity 8.177 GBq/mmol). This six step synthesis from [^{3}H]$NaBH_4$ gave an overall yield of 6%. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: tritium; anxiolytic agents* 1*R*,2*S*,4*R*-(–)-2-(dimethylaminoethoxy)-2-phenyl-1,7,7-trimethylbicyclo[2.2.1]heptane; [3-^{3}H]deramciclane; [2-dimethylamino-[2-^{3}H]ethoxy]deramciclane

Introduction

Deramciclane fumarate (EGIS-3886) (Figure 1) is a new putative non-benzodiazepin-type anxiolytic compound, synthesized by EGIS Pharmaceutical Ltd.[1]

Deramciclane possesses high affinity to 5-HT_2 receptors, where it exerts antagonistic effects.[2–4] Previously published pharmacokinetic studies in several

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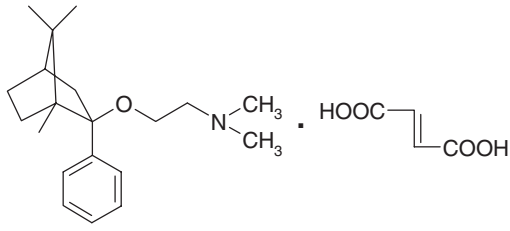


Deramciclane fumarate

Figure 1. Deramciclane fumarate

different species, including man, have shown extensive metabolism, with cleavage of the side chain the major metabolic pathway.[5–10] The resulting metabolite and derivatives show increased distribution and significant plasma levels for a longer biological half-life than the parent compound.[11,12] Two tritium labelled forms of deramciclane were required with the label in the camphor skeleton, ([3-^{3}H]deramciclane (**3**)) and the side chain ([2-dimethylamino-[2-^{3}H]ethoxy]deramciclane (**9**)) for *in vitro* and *in vivo* studies to further investigate various aspects of the metabolism and pharmacokinetics of deramciclane.[11–19] Previous studies with ^{3}H and ^{14}C labelled deramciclane had showed that the metabolism of the camphor skeleton and side chain could be differentiated by virtue of their significantly different biological half-life values.[11]

Discussion

Preliminary '*in vitro*' studies of the biotransformation of deramciclane required the synthesis of two separate forms of tritium labelled deramciclane, in order to fully trace the fate of the camphor skeleton and the side chain. Hence using synthetic methodology supplied by EGIS Pharmaceuticals Ltd. [3-^{3}H]deramciclane (**3**) and [2-dimethylamino-[2-^{3}H]ethoxy]deramciclane (**9**) were prepared.

The synthesis of [3-^{3}H]deramciclane was realized as shown in Figure 2. [3-^{3}H]camphor was prepared from [(1*R*)-*endo*]-(+)-3-bromocamphor by tritiation with $^3H_2$ in the presence of Pd/C catalyst in dioxane. The remainder of the synthesis was carried out using the procedure[2] described earlier for unlabelled deramciclane. The [3-^{3}H]camphor (**1**) was condensed with phenyl magnesium bromide in diethylether giving rise to [3-^{3}H]phenylborneol (**2**). The latter, after conversion into its sodium salt, was reacted with dimethylaminoethyl chloride to give [3-^{3}H]deramciclane (**3**), which was isolated as the fumarate salt.

For the synthesis of [2-dimethylamino-[2-^{3}H]ethoxy]deramciclane (see Figure 3), the benzyloxy-acetic acid methyl ester was reduced with

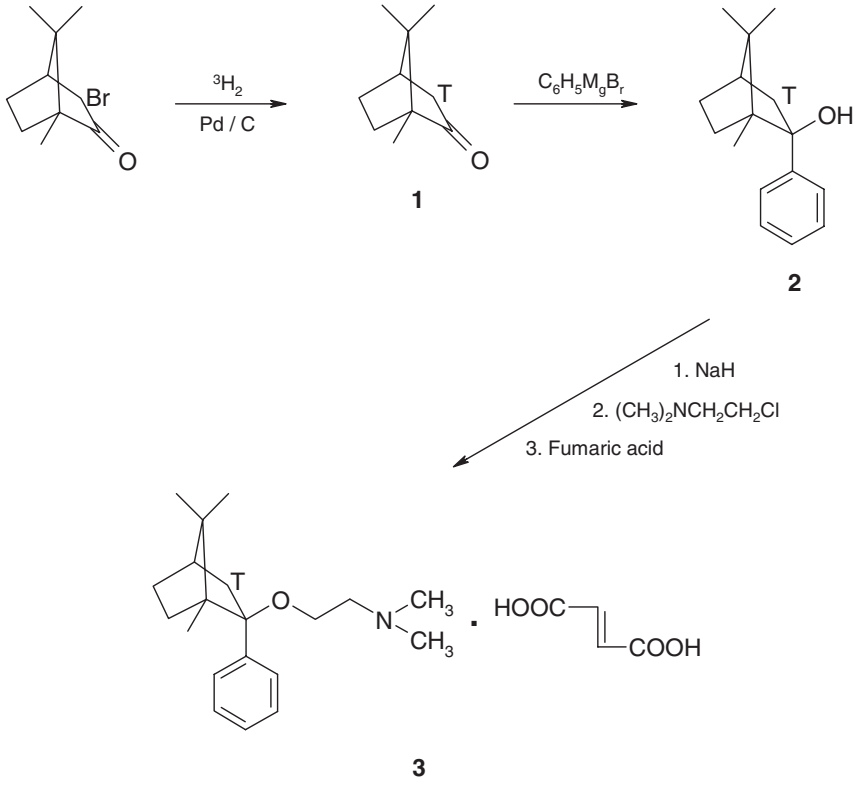


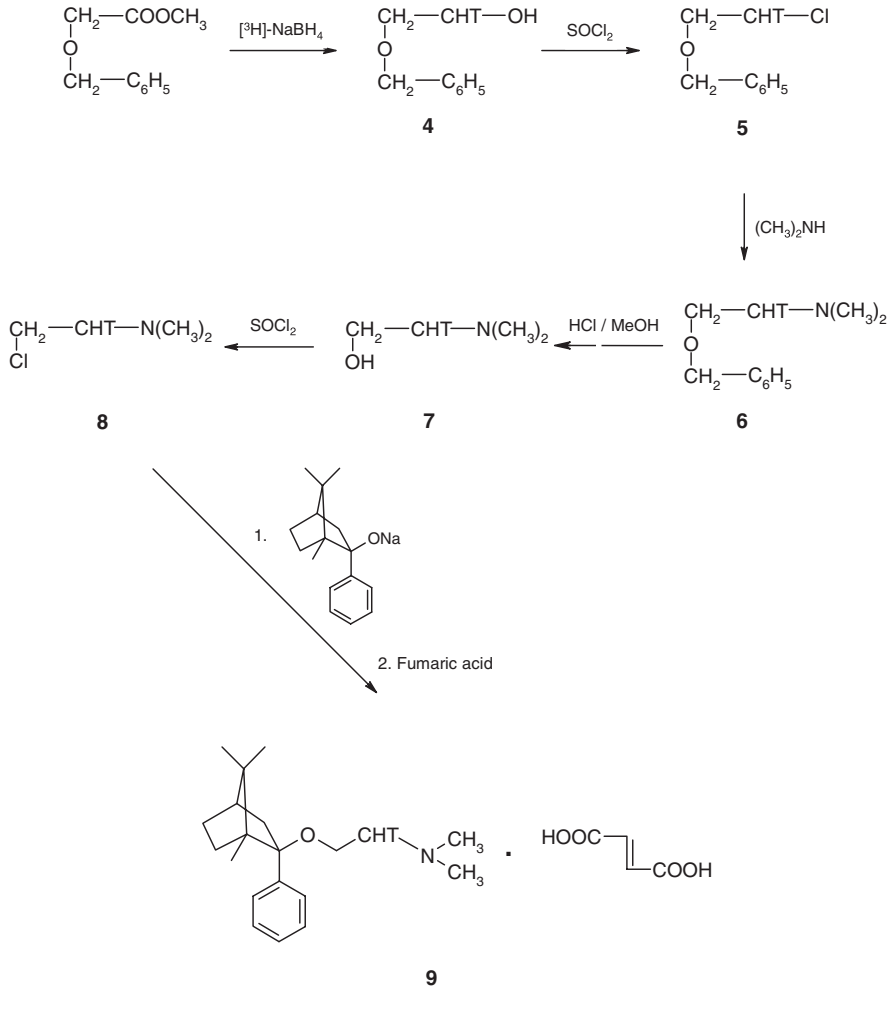
Figure 2. [3-^{3}H]-Deramciclane fumarate

sodium-borotritide to 2-benzyloxy-ethanol-[1-^{3}H] (**4**) and then converted to 2-benzyloxy-ethylchloride-[1-^{3}H] (**5**) with thionyl chloride. The **5** was aminated with dimethylamine to 2-benzyloxy-1-dimethylamino-ethane-[1-^{3}H] (**6**) and then hydrolyzed to 2-dimethylaminoethanol-[2-^{3}H] (**7**), which was chlorinated with thionyl chloride to 2-dimethylaminoethyl-[2-^{3}H] chloride (**8**). It was condensed with the sodium derivative of 2-phenylborneol giving rise to [2-dimethylamino-[2-^{3}H]ethoxy]deramciclane (**9**) isolated as the fumarate.

Experimental

Materials

All reagents were analytical grade and used without further purification. Tritium gas and sodium-borotritide were purchased from the Institute of Isotopes Ltd. (Budapest, Hungary). Column chromatography was performed on silica gel 60 (0.063–0.200 mm, Merck).



[2-dimethylamino-[2-^{3}H]ethoxy]deramciclane fumarate

Figure 3. [2-dimethylamino-[2-^{3}H]ethoxy]deramciclane

Synthesis

*[3-^{3}H]phenylborneol (**2**)*. In the presence of 10% Pd/C (5 mg) catalyst the [(1*R*)-*endo*]-(+)-3-bromocamphor (10 mg) was hydrogenated with tritium gas in 1 ml of dioxane for 3 h using a tritium manifold system. To the dioxane solution of [3-^{3}H]camphor (**1**) formed in the reaction, 5 ml of diethylether containing 40 mg of unlabelled camphor was added. This solution was added under an argon atmosphere to the Grignard solution prepared from 80 mg of bromobenzene and 12 mg of Mg in 5 ml of ether. The reaction mixture was stirred at room temperature for 1 h then refluxed for 30 min. The cooled reaction mixture was quenched with water and acidified with conc.

hydrochloric acid. The layers were separated and the aqueous phase was extracted with ether. The organic phase was dried, evaporated and the crude [3-^{3}H]phenylborneol (**2**) was purified by column chromatography (eluent: ether–hexane = 1:1).

*[3-^{3}H]-1R, 2S, 4R-(−)-2-(dimethylaminoethoxy)-2-phenyl-1, 7, 7-trimethylbicyclo [2.2.1] heptane (deramciclane) (**3**)*. The pure [3-^{3}H]phenylborneol (35 mg, 7.6 GBq) was converted into its sodium salt by addition of 20 mg of NaH (55% in mineral oil suspension) and refluxing in toluene (3 ml) for 2 h under an argon atmosphere. To this reaction mixture 60 mg of dimethylaminoethyl chloride was added in 2 ml of toluene and the reaction refluxed for 3 h. After cooling it was evaporated to dryness and the product was purified by column chromatography (eluent: chloroform–methanol–ammonia-sol. = 95:5:0.5) to give pure [3-^{3}H]deramciclane (**3**), which was isolated as the fumarate salt. This three step synthesis from [3-^{3}H]camphor gave an overall yield of 22%. The specific radioactivity was 51.8 GBq/mmol and the radiochemical purity was better than 98%.[20]

*2-Dimethylaminoethyl-[2-^{3}H] chloride hydrochloride (**8**)*. To the stirred reaction mixture containing benzyloxy-acetic acid methyl ester (20 mg) and sodium-borotritide (3.7 GBq, 6 mg) in *tert*-butanol (2 ml), 0.2 ml of methanol was added at reflux temperature and was further refluxed for 1 h. The solvent was distilled off and the residue (2-benzyloxy-ethanol-[1-^{3}H] (**4**)) was dissolved in chloroform, and purified by column chromatography (eluent: benzene–methanol = 8:2). The isolated pure **4** (2.8 GBq) was then refluxed with 0.5 ml of thionyl chloride for 30 min and then evaporated to dryness. The formed crude 2-benzyloxy-ethylchloride-[1-^{3}H] (**5**) was dissolved in 1 ml of methanol, transferred to a glass tube and 0.1 mmol of dimethylamine was added. The tube was sealed and warmed at 80°C for 1 h resulting in the formation of 2-benzyloxy-1-dimethylamino-ethane-[1-^{3}H] (**6**). After allowing to cool, and opening the tube, 10 ml of 2 N hydrochloric acid was added to the methanolic solution of **6** and the reaction mixture was refluxed for 1 h. The solvent was then distilled off and the residue was rendered alkaline with 40% sodium hydroxide and steam-distilled. About 50 ml of distillate was collected, acidified with hydrochloric acid and evaporated. The obtained 2-dimethylaminoethanol-[2-^{3}H] hydrochloride (**7**) was refluxed with thionyl chloride (1 ml) for 30 min then evaporated, resulting in 2-dimethylaminoethyl-[2-^{3}H] chloride hydrochloride (**8**) (405 MBq), which was used directly in the next step.

*1R, 2S, 4R-(−)-2-(2-dimethylamino-2-[^{3}H]ethoxy)-2-phenyl-1, 7, 7-trimethylbicyclo [2.2.1] heptane (deramciclane) (**9**)*. The water solution of **8** was made alkaline with 50% sodium hydroxide solution and extracted with 3 × 4 ml of toluene. The toluene phase was dried with potassium carbonate, filtered and

concentrated to about 3 ml and condensed with the sodium derivative of 2-phenylborneol as previously described giving rise to [2-dimethylamino-[2-^{3}H]ethoxy]deramciclane (**9**) isolated as the fumarate (222 MBq). This six step synthesis from [^{3}H]$NaBH_4$ gave an overall yield of 6%. The specific radioactivity was 8.177 GBq/mmol and the chemical and radiochemical purity were greater than 98%.[16]

Analysis

Radioactivity measurements and determination of chemical and radiochemical purity of [3-^{3}H]deramciclane and [2-dimethylamino-[2-^{3}H]ethoxy]deramciclane were performed by the following methods.

Chemical and radiochemical purity of the products were checked by thin layer/overpressured layer – (TLC/OPLC), high-performance liquid – (HPLC) and gas chromatography, on the basis of comparison with deramciclane standard.

All chemicals and solvents were of analytical or HPLC-solvent grade.

Radioactivity of samples was measured by liquid scintillation with RackBeta Liquid Scintillation Counter Model 1217-001 (LKB-Wallac, Turku, Finland) in a cocktail of benzyl alcohol (1000 ml)–ethanol (200 ml) and PPO (6 g).

Thin layer chromatography. TLC-sheet: DC Alufolien Kieselgel 60 F_{254} or HPTLC layer (Merck, Darmstadt).

Eluent: chloroform:methanol:ammonia-sol. = 95:5:0.5.

Detection: UV light and Digital Autoradiograph (DAR) [(EG&G Berthold LB 287, WinDAR Software – Vildbad, Germany), run time: 30 min, gas flow: 5 ml/min, counter voltage: 2040 V].[13,15,20,21]

Overpressured layer chromatography. OPLC separation: Aluminium-backed HPTLC Kiselgel 60 F_{254} layer (Merck, Darmstadt) (20 × 20 cm; 0.20 mm; sealed on all four edges).

OPLC system: OPLC 50 Instrument (optimum-performance laminar chromatography) Bionisis SA (Le Plessis-Robinson, France).

Mobile phase: 1-butanol–acetic acid–water (6:1:1 v/v/v);
external pressure 50 Mpa;
flow rate 250 µl/ml.

Detection: Digital Autoradiography (DAR) [(EG&G Berthold LB 287, WinDAR Software – Vildbad, Germany), run time: 30 min, gas flow: 5 ml/min, counter voltage: 2040 V].[13,15,20,21]

High-performance liquid chromatography. ISCO HPLC-system (Model 2360 Gradient Programmer, Model 2350 HPLC-Pump, V^4 Variable Wavelength Absorbance Detector; Lincoln, Nebraska, USA) was used under the following conditions:

Column: Hypersil ODS (5 μm, 250 × 4 mm ID).

Mobile phase: 60% acetonitrile – 40% water adjusted to pH3 with [0.1% (70% perchloric acid) and triethylamine].

Detection: At 220 nm.

Radioactivity was measured by a HPLC-Radioactivity Monitor (Hewlett-Packard 1090M-EG&G Berthold LB506-C-1), parameters: Scintillator cocktail Quickscint Flow 301 (Zinsser Analytic), detector cell type: Z 1000 **4** (1 ml).

Gas chromatography. Hewlett-Packard 5890 Series II gas chromatograph (HP, Palo Alto, USA) connected to Raytest RAGA-93 Radioactivity Gas Analyzer (Raytest Isotopenmessgerate GmbH, Straubenhardt, Germany). The reactor of the RAYTEST RAGA-93 radioactive gas analyser (detector; RD) was connected to the capillary column in the oven of the gas chromatograph. The packing of the reactor consisted of approximately 6 g of platinum wire shavings. The packing was placed into the 2 mm ID, 165 mm long fused silica reactor tube so that it was located in the middle third of the catalyst. Platinum shavings were fixed with asbestos wool. Reduction was carried out at 750°C in 8 ml/min hydrogen stream. High purity (4.5) methane at a 15 ml/min flow rate was used as counter gas. The proportional counter was operated at 3400 V and the splitter was set to 100%.

GC column: SUPELCO SPB-5 (30 m × 0.25 mm, film thickness: 0.25 μm).

Temperature:	100°C (0.4 min), 13°C/min: 185°C (20 min), 50°C/min: 250°C (4 min), 70°C/min: 100°C.
Carrier (He):	340 kPa (0.05 min), 500 kPa/min: 190 kPa (7 min), 400 kPa/min: 100 kPa (21 min), 100 kPa/min: 340 kPa.
Injector:	Split (10 ml/min), 240°C.
Injected vol.:	2 μl.

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