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# CARBON-14 LABELLING OF TERBINAFINE, AN ANTIMYCOTIC AGENT

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## Summary

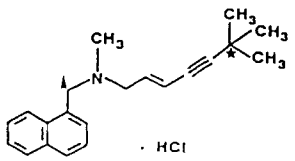
Terbinafine is a new antimycotic agent which is currently being tested worldwide in clinical trials.
The synthesis of [$^{14}$C]Terbinafine, labelled in the naphthalene methane moiety as well as in the tertiary butyl group are described. The key step of the latter is the aluminium-mediated tertiary butylation of an alkyne.

Key words : terbinafine, antimycotic agent, carbon-14 labelling, aluminium-mediated tertiary butylation, [$^{14}$C]-t-butanol

## Introduction

Allylamine derivatives are a new class of antimycotic agents. They act as potent, selective inhibitors of fungal squalene epoxidase [1].
The highly potent compound (E)-N-(6,6-dimethyl-2-hepten-4-ynyl)-N-methyl-1-naphthalene-methanamine hydrochloride, Terbinafine, was labelled with carbon-14 in order to study its pharmacokinetics and biotransformation in animals and in man.
The present paper describes the synthesis of two differently carbon-14 labelled Terbinafines (4), containing the label either in the naphthalene methane moiety (Δ) or in the tert. butyl group (*) (see Figure 1).

Figure 1

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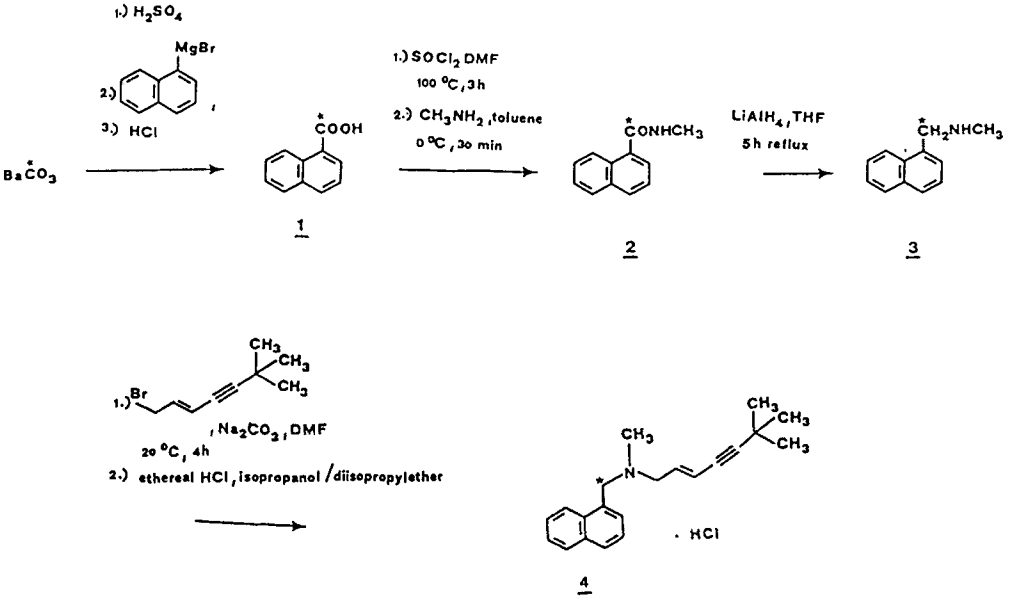
## Results and Discussion

**1. Labelling with carbon-14 in the naphthalene methane moiety (Scheme 1).**

Scheme 1



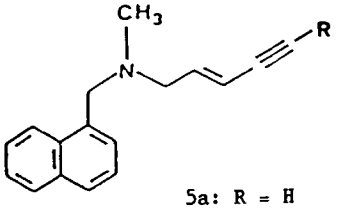

The label was introduced by carboxylation of 1-naphthylmagnesium bromide with [$^{14}$C]carbon dioxide in dry ether according to standard procedures [2]. The resulting 1-[carboxyl-$^{14}$C]naphthoic acid 1 was refluxed with thionyl chloride in the presence of catalytic amounts of dimethylformamide for 3 h. After removal of all volatile material under reduced pressure the crude 1-[carbonyl-$^{14}$C]naphthoyl chloride was dissolved in toluene and treated with methylamine at 0°C for 30 min. The N-methyl-1-naphthalene [$^{14}$C]carboxamide 2 obtained was reduced to N-methyl-N-1-naphthalene[$^{14}$C]methanamine 3 with lithium aluminium hydride in refluxing tetrahydrofuran.

In the last step of the reaction sequence 3 was alkylated with E/Z-1-bromo-6,6-dimethyl-2-hepten-4-yne (E > 95 %) in dimethylformamide (20°C, 16 h). The resulting crude [$^{14}$C]SF 86-327-b was purified by column chromatography on silica gel with methylene chloride - methanol 99:1, identified by radio-TLC, $^{1}$H-nmr and mass-spectroscopy, and adjusted to a suitable specific activity by addition of unlabelled carrier. Finally, it was dissolved in isopropanol and converted to the hydrochloride with ethereal HCl.

**2. Labelling in the t-butyl group**

The first metabolic studies on Terbinafine labelled with C-14 in the naphthalene methane moiety revealed that a major metabolic pathway was the cleavage of the side-chain of the molecule. In order to obtain detailed information about the metabolic fate of the side-chain, the label was introduced into the t-butyl group.

It seemed to be logical to try to attach a labelled t-butyl group to the corresponding unsubstituted acetylenic amine 5a.



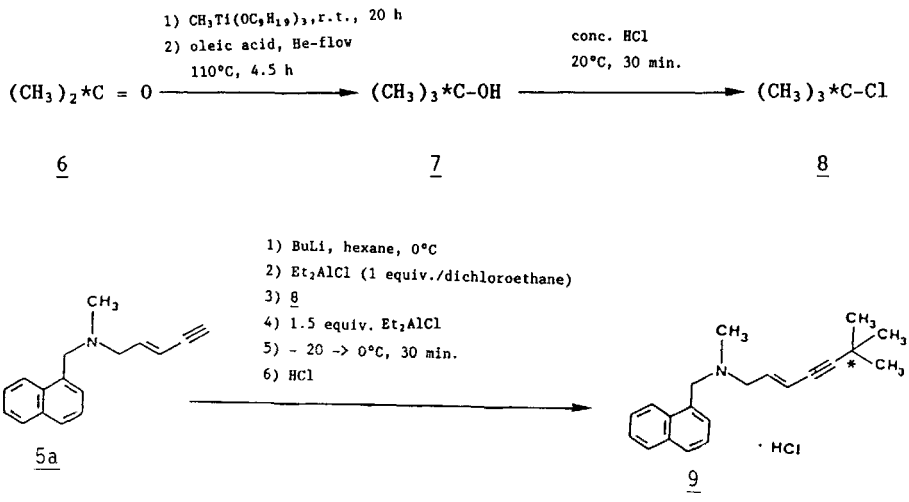

5a: R = H
5b: R = 1/3 Al
5c: R = $AlEt_2$

The only hitherto known satisfactory method for tertiary alkylation of alkynes with electrophiles is the reaction of trialkynyl aluminium compounds with tertiary alkyl halides or mesylates [3]. When applied to Terbinafine the trialkynyl aluminium compound 5b proved to be unreactive with t -butyl chloride. Addition of an equivalent of aluminium chloride in order to complex the free amine function resulted in a rapid reaction and formation of several products, among them the desired Terbinafine. However, the yield was low and the product was difficult to isolate.

Much better results were obtained when the corresponding diethylaluminium alkynyl compound 5c was used. The acetylenic amine 5a was converted to its C-lithio derivative and subsequently quenched with 1 equiv. of diethylaluminium chloride. Addition of 1 equiv. of t -butyl chloride followed by another 2.5 equiv. of diethylaluminium chloride at - 20°C to r.t. resulted in a clean formation of the desired t-butylated product in 60 % yield. The hot experiment, however, gave only 35 % isolated yield, probably due to the quality of the [$^{14}C$]t-butyl chloride (see below). The whole synthetic pathway providing [$^{14}C$]Terbinafine is outlined in Scheme 2:

[2-$^{14}C$]Acetone (6) was prepared via barium [$^{14}C$]acetate according to the literature [4]. Addition of the neat non-basic tris-nonyloxy methyl titanium [5] to [$^{14}C$]acetone was carried out at room temperature under stirring for two hours. Liberation of the formed 2-methyl[2-$^{14}C$]propan-2-ol (7) was effected by oleic acid. The use of non-volatile reagents facilitated a convenient isolation of the volatile product. 7 was converted to 2-chloro-2-methyl[2-$^{14}C$]propane (8)

Scheme 2




([$^{14}$C]t-butyl chloride) by stirring with conc. hydrochloric acid at room temperature for 30 min. Whereas the formation of cold t-butyl chloride proceeds without problems, hot (50-60 mCi/mmol)t-butanol probably also forms the elimination product isobutene which gives rise to lower yields in the next step. 8 finally reacted with the aluminium reagent 5c as described above to give the desired [$^{14}$C]Terbinafine (9) in a fair yield together with unchanged starting material.

It is noteworthy that the tertiary alkylation of an acetylene could be accomplished in the presence of other functionalities as tertiary amine and an aromatic nucleus by the use of diethylaluminium chloride as Lewis acid as well as reagent for the formation of the intermediate aluminium reagent 5c. Despite the somewhat low yield, the described procedure presents a convenient access to tertiary alkyl acetylenes bearing further functionalities.

## Experimental

$^1$H-nmr spectra were recorded on a Bruker AM 360 apparatus operating in the Fourier transform technique.

Mass spectra were recorded on a MAT 212 apparatus. Radiochemical purity was determined by scanning silica gel 60 F254 TLC plates (Merck) using either a Berthold Dünnschichtscanner II or a Berthold TLC Linear Analyzer LB 283.

Radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer (Model 3375).

1-[carboxyl-$^{14}$C]Naphthoic acid (1)

[$^{14}$C]Carbon dioxide prepared from 987 mg (5 mmol) of barium [$^{14}$C]carbonate (290 mCi totally) was vacuum-transferred to the frozen Grignard reagent, prepared from 288 mg (12 matom) of magnesium turnings and 21 g (10 mmol) of 1-bromonaphthalene in 20 ml of anhydrous tetrahydrofuran. The reaction mixture was stirred for 1 h at - 10°C, then decomposed by addition of 2-3 ml of 2 N HCl and worked up extractively in the normal manner. The obtained acid (780 mg, 4.53 mmol = 90.6 % yield) proved to have a radiochemical purity of > 98 % as determined by TLC on silica gel 60 F254 with methylene chloride - methanol 98:2 as solvent system.

The identity of the compound was confirmed by TLC and melting point determination with an authentic sample of the pilot run at the tracer level. It was used for the following sequence without further purification.

N-Methyl-1-naphthalene[$^{14}$C]carboxamide (2)

780 mg (4.53 mmol, ca. 260 mCi) of 1-[carboxyl-$^{14}$C]naphthoic acid were dissolved in 15 ml of freshly distilled thionyl chloride under anhydrous conditions. After addition of 2 drops of dry dimethylformamide the reaction mixture was heated to 70°C for 2 h and subsequently evaporated to dryness. The residue was dried in vacuo at room temperature for 30 min, dissolved in 30 ml of dry toluene and cooled to 0°C. A stream of dry methylamine was bubbled for 30 min through the acid chloride solution, the resulting suspension was poured onto ice, and the crude N-methyl-1-naphthalene[$^{14}$C]carboxamide formed was isolated by repeated extraction with methylene chloride - isopropanol 3:1. The combined organic layers were washed with NaCl solution and dried over anhydrous $Na_2SO_4$. The solvent was removed under reduced pressure. The residue consisted of radiochemically pure (> 98 %) amide 2 (831 mg, 99 %) as determined by TLC (silica gel 60 F254 (Merck); methylene chloride - methanol 98:2 , UV (254 nm).

N-Methyl-N-1-naphthalene[$^{14}$C]methanamine hydrochloride (3)

A solution of 831 mg (4.48 mmol) of N-methyl-1-naphthalene[$^{14}$C]carboxamide 2 in 10 ml of dry tetrahydrofuran was added dropwise to a stirred suspension of 300 mg of lithium aluminium hydride in 20 ml of dry tetrahydrofurane. The mixture was refluxed overnight, cooled to room temperature, cautiously decomposed with 10 ml of saturated $Na_2SO_4$ solution and diluted with ether. The precipitated aluminium hydroxide was dissolved by addition of aqueous potassium hydroxide solution. The aqueous layer was extracted repeatedly with ether and the

combined organic extracts were then washed with 2 N HCl. The acidic washings were evaporated to dryness to give 1016 mg (109 %) of crude amine-hydrochloride 3, which was recrystallised once from ethanol - ether yielding 757.6 mg of pure 3 (212 mCi, 3.65 mmol, 81.4 %).

The mother liquor was chromatographed over silica gel 60 (methylene chloride - methanol - conc. ammonia 90:10:1) to give 49 mg (0.286 mmol, 16.6 mCi) of free amine 3. The radiochemical purity of the two crops was 98 % as determined by TLC (silica gel 60 F254; methylene chloride - ethanol - conc. ammonia 84:15:1 or ethylacetate - 2-butanone - methanol - water - formic acid 60:40:15:15:1 or methylene chloride - methanol 8:2), UV (254 nm), iodine vapour.

[$^{14}$C]Terbinafine (4)

To a stirred mixture of 107.5 mg (0.628 mmol), 36.5 mCi) of the amine 3, 300 mg of sodium carbonate and 3 ml of dimethylformamide were added 150 mg (0.75 mmol) of 1-bromo-6,6-dimethyl-2-hepten-4-yne as an E/Z-mixture (> 95 % E) dissolved in 600 µl DMF. The mixture was stirred for 4h, dissolved in water and extracted with ether. The ethereal solution was washed with water, evaporated, and the residue was chromatographed over silica gel using methylene chloride- isopropanol 95:5 as eluant.

The fractions containing product were evaporated, the resulting oily amine taken up with methylene chloride containing a few drops of ethanolic hydrogen chloride and again evaporated.

Recrystallisation from isopropanol - diisopropylether afforded 159 mg (77 %) of colourless crystals, which were subsequently recrystallised after addition of 160 mg of cold material to give 276 mg of [$^{14}$C]Terbinafine hydrochloride (0.84 mmol, 85 µCi/mg, 27.9 mCi/mmol, 23.5 mCi totally). The radiochemical purity (> 98 %) was determined by TLC/radio-TLC on silica gel 60 F254 using the following solvent systems: ethyl acetate - 2-butanone - water - methanol - formic acid 60:40:10:10:1 ; hexane - methyl acetate - methylene chloride - conc. ammonia 60:20:20:1; methylene chloride - methanol 95:5.

The analysis of the mass spectrum confirmed the identity and the 1:1.2 ratio of [$^{14}$C]Terbinafine and unlabelled Terbinafine. The nmr-spectra of labelled and unlabelled Terbinafine were superimposable.

[2-$^{14}$C]Acetone (6)

[2-$^{14}$C]Acetone was prepared from 10 mmoles of barium[$^{14}$C]carbonate (ca. 55 mCi/mmol) via barium[$^{14}$C]acetate and pyrolysis of the latter according to the literature [4].

Yield : first run : 270 mg (93.1 % based on barium[$^{14}$C]carbonate)
second run : 276 mg (95.0 % based on barium[$^{14}$C]carbonate)

2-Methyl[2-$^{14}$C]propan-2-ol (7)

5.41 g (10.1 mmol) of neat tris-nonyloxy methyl titanium [5] were placed in a two-necked round bottom flask and evacuated. 270 mg (4.65 mmol) of [2-$^{14}$C]acetone were condensed onto the reagent and the mixture was stirred at room temperature for 30 min. Then the vacuum was released with argon and stirring was continued overnight at room temperature. Oleic acid (5.65 ml, 5.03 g, 17.8 mmol) was added and the mixture was heated to 110°C. The product was transferred to a cooled trap with a slow stream of helium over 4.5 hours. From this trap it was condensed into a break seal ampoule under high vacuum. Yield; 360 mg, 4.86 mmol, 104 %. In a second run starting from 276 mg of [$^{14}$C]acetone, 315 mg (89.5 %) of 7 were obtained.

The product solidified only at temperatures below 0°C indicating some impurities. The same melting point depression was observed in the cold runs where the nmr-spectra of the product revealed a purity of ~ 95 %.

2-Chloro-2-methyl[2-$^{14}$C]propane (8)

2.5 ml of conc. hydrochloric acid (37 %) were placed in a two-necked flask equipped with a gas-inlet and a reflux condenser. 360 mg (4.86 mmol) of 7 were condensed onto the hydrochloric acid at - 196°C. The mixture was allowed to warm up to room temperature and stirred for one hour. The product was transferred with helium into a trap cooled with liquid nitrogen. From this trap the product was transferred into a break-seal ampoule through a drying tube containing a zone with calcium chloride and another zone with sodium hydroxide on a mineral carrier.
Yield : 313 mg (69.6 %).

In a second run starting from 315 mg of 7, 220 mg of 3 (55.9 %) were obtained giving a total yield of 533 mg (5.76 mmol). The product contained variable amounts of a more volatile impurity, probably isobutene.

[$^{14}$C]Terbinafine (9)

1.35 g (5.76 mmol) of (E)-N-2-penten-4-ynyl)-N-methyl-1-naphthalenemethanamine in 8.5 ml of dry hexane were placed in a two-necked flask under argon. At - 20°C, 3.6 ml of 1.6 N butyllithium (5.76 mmol) were added through a septum via syringe and the precipitate was stirred for 5 minutes. Then 5.76 ml of a 1 M solution of diethylaluminium chloride in hexane were added at the same temperature followed by 22.8 ml of dichloroethane. The nearly transparent solution

was then cooled to - 196°C, evacuated and 533 mg of crude 8 were condensed onto the reaction mixture. The flask was immersed in an ice-salt mixture and the reaction mixture was stirred for 30 minutes before another equivalent (5.76 ml) of diethylaluminium chloride solution was added. The mixture was stirred 30 min at - 20°C and 30 min at room temperature. Finally, an additional 3 ml of the diethylaluminium chloride solution were added and stirring was continued at room temperature for 2 hours.

5 ml of water were added under cooling with ice and all volatile components were lyophilised off. The residue was partitioned between methylene chloride - isopropanol 9:1 and 2 N NaOH; the solvent was evaporated and the crude product (1683 mg) was chromatographed on silica gel M 0.015 - 0.04 mm (Merck) using n-hexane - butylacetate 97:5:2.5 as eluent to obtain 630 mg of [$^{14}$C]Terbinafine-base. The base was converted into its hydrochloride by dissolving in methylene chloride (3 ml) and addition of 1 ml of methanolic hydrogen chloride, evaporation of the solvent, redissolving in 2 ml of isopropanol and addition of 7.5 ml of diisopropyl-ether. The mixture was allowed to stand in a refrigerator overnight for crystallisation. The crystals were washed with diisopropyl ether and dried under high vacuum.

Repetition of the crystallisation yielded 664 mg (2.025 mmol) of pure [$^{14}$C]Terbinafine hydrochloride 9 (35.2 % based on 8).

Radiochemical purity (TLC): $\geq$ 98 %.

Specific activity: $3.75 \times 10^8$ dpm/mg = 168.9 µCi/mg = 55.4 mCi/mmol.

Mass spectrum: The 87:13 ratio (= 54.3 mCi/mmol) of labelled Terbinafine and unlabelled Terbinafine was in good agreement with the measured specific activity of 55.4 mCi/mmol determined by LSC.

NMR : The spectra of labelled and unlabelled Terbinafine were superimposable.

Acknowledgment

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