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Research Article

Microbial synthesis of a deuterium-labelled metabolite of an NK1 receptor antagonist, TAK-637

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Summary

Microbial conversion of deuterium-labelled TAK-637 ((*aR,9S*)-[10,10,11,11-$^2H_4$]-7-[3,5-*bis*(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-7*H*-[1,4]diazocino[2,1-*g*][1,7]naphthyridine-6,13-dione, **1**) was carried out using *Streptomyces subrutilus* IFO13388 to give a deuterium-labelled hydroxylated TAK-637 ((*aR,9S*)- [10,10,11,11-$^2H_4$]-7-[3,5-*bis*(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-hydroxymethylphenyl)-7*H*-[1,4]diazocino[2,1-*g*][1,7]naphthyridine-6,13-dione, **2**) for use as an internal standard in LC-MS studies. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: *Streptomyces subrutilus* IFO13388; Microbial conversion; Deuterium labelling

Introduction

The study of drug metabolism is becoming an increasingly important aspect of the drug development process. Deuterium-labelled metabolites are required for use as an internal standard for drug metabolism studies and the chemical syntheses of such metabolites have been reported.[1–5]

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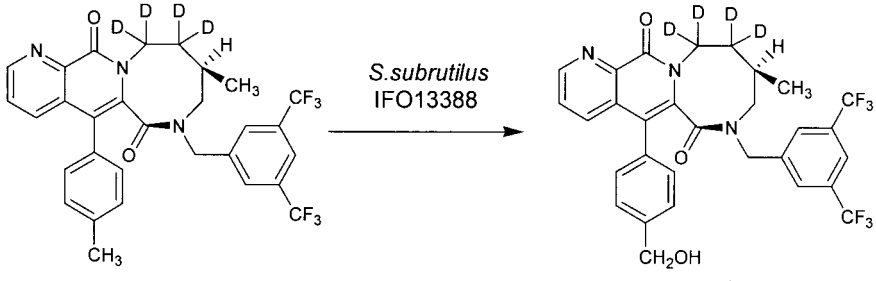
However, there have been only a few reports describing the synthesis of deuterium-labelled metabolites using microorganisms.

The compound TAK-637 ((*aR,9R*)-7-[3,5-*bis*(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-*7H*-[1,4]diazocino [2,1-*g*][1,7]naphthyridine-6,13-dione), an NK1 receptor antagonist for the treatment of bladder function disorders, has been observed to be hydroxylated in humans. Deuterium-labelled hydroxylated TAK-637 ((*aR,9S*)-[10,10,11,11-$^2H_4$]-7-[3,5-*bis*(trifluoromethyl)benzy1]-8,9,10,11-tetrahydro-9-methyl-5-(4-hydroxymethylphenyl)-*7H*-[1,4]diazocino[2,1-*g*] [1,7]naphthyridine-6,13-dione, d_4-hydroxylated TAK-637, **2**) was required for use as an internal standard in an LC-MS assay for TAK-637 metabolism. It was difficult, however, to obtain a large amount of the d_4-hydroxylated TAK-637 by chemical synthesis. We have found that TAK-637 was converted to the hydroxylated TAK-637 by actinomycetes,[6] and we applied the microbial conversion for the synthesis of d_4-hydroxylated TAK-637 (Scheme 1).



1 **2**

Scheme 1.

Results and discussion

We synthesized deuterium-labelled TAK-637((*aR,9S*)- [10,10,11, 11-$^2H_4$]-7-[3,5-*bis*(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-*7H*-[1,4]diazocino[2,1-*g*][1,7]naphthyridine-6,13-dione, d_4-TAK-637, **1**) using (*S*)- [3,3,4,4-$^2H_4$]-4-amino-2-methyl-1-butanol THP ether in place of (*R*)-4-amino-2-methyl-1-butanol THP ether according to a procedure similar to that described previously.[7]

Microbial conversion of d_4-TAK-637 was carried out to obtain d_4-hydroxylated TAK-637. HPLC analysis showed that d_4-hydroxy-

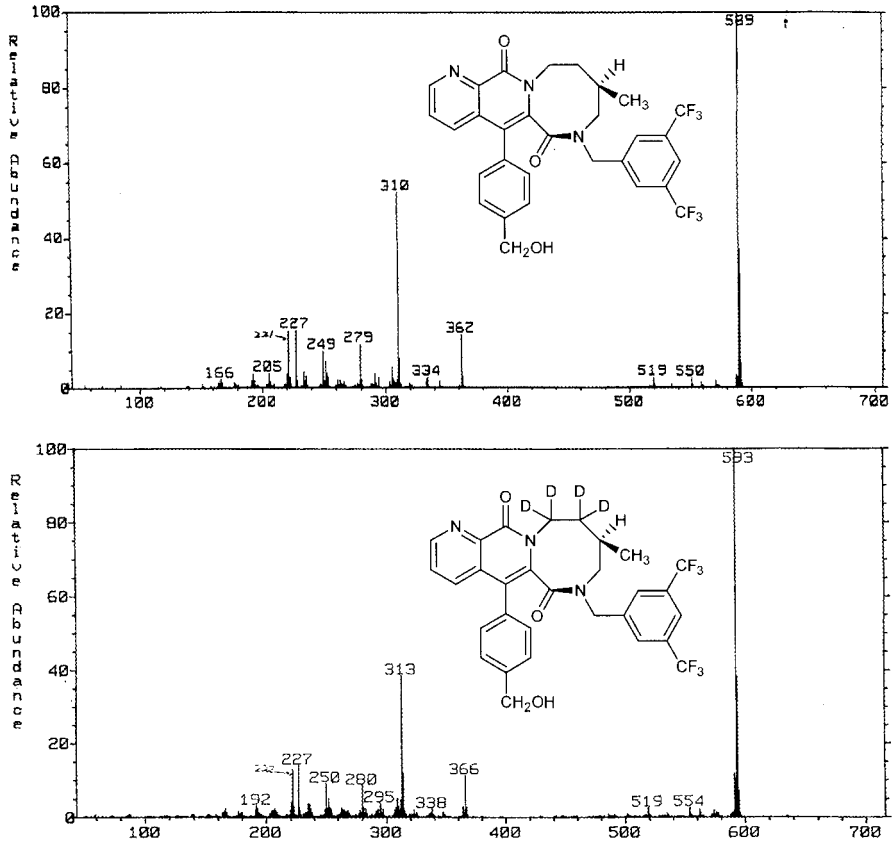


Figure 1. Mass spectrum of d_4-hydroxylated TAK-637 (bottom) compared with mass spectrum of hydroxylated TAK-637 (top)

lated TAK-637 had a retention time of 5.3 min. The retention time of d_4-hydroxylated TAK-637 was consistent with that of hydroxylated TAK-637. As for the location of the four $^2H_4$ atoms in d_4-hydroxylated TAK-637, it was readily apparent in the ^{1}H NMR spectrum that signals had undergone a change compared to the spectrum of the hydroxylated TAK-637.[6] Specifically, the signals for the four protons (1.5–1.9 (1H, m), 1.95–2.40 (1H, m), 3.35–3.65 (1H, m), 5.10 (1H, dd, $J = 15$, 5.5 Hz)) disappeared. Furthermore, the mass spectra of M^+ ions of the d_4-hydroxylated TAK-637 and the hydroxylated TAK-637 at m/z 593($= 589 + 4$) and m/z 589 confirmed the structure (Figure 1).

Experimental

Streptomyces subrutilus IFO13388, used in this study, was obtained from the Institute for Fermentation, Osaka (IFO). The medium (pH 7) contained 5 g/l glucose, 50 g/l dextrin, 35 g/l soybean meal, and 7 g/l $CaCO_3$. HPLC analysis was conducted using an L7100 system (Hitachi, Ltd., Japan) and UV detection at 310 nm. The mobile phase consisted of an aqueous 10 mM KH_2PO_4 and acetonitrile (1 : 1). A Develosil ODS UG-5 column (Nomura Chemical, Ltd., Japan) was used and the solvent flow rate was 1.0 ml/min. Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H NMR spectra were taken on a Varian Gemini 200 (200 MHz) spectrometer in $CDCl_3$. Chemical shifts are given in ppm with tetramethylsilane as the internal standard, and coupling constants (J) are given in hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, m = multiple, dd = double doublet. Mass spectra were obtained on a JEOL JMS-AX505W spectrometer. Elemental analyses were carried out by Takeda Analytical Research Laboratories, Ltd.

(S)-[3,3,4,4-$^2H_4$]-4-amino-2-methyl-1-butanol tetrahydropyranyl (THP) ether

To a stirred suspension of $LiAl^2H_4$ (5.80 g) in ethyl ether (200 ml), a solution of (*S*)-(+)-3-hydroxy-2-methyl-1-propanol THP ether (39.5 g)(8) in ethyl ether (60 ml) was added dropwise at 0°C and the mixture was stirred at room temperature for 1 h and cooled on ice. To the mixture, 1 N NaOH (24 ml) and tetrahydrofuran (THF) (24 ml) were added with stirring. The mixture was filtered through a Celite pad. The filtrate was dried and evaporated to give (*S*)-[1,1-$^2H_4$]-2-methyl-1,3-propanediol mono THP ether as a colorless oil (35.6 g). To a solution of the colorless oil (35.6 g) in pyridine (150 ml), *p*-toluenesulfonyl chloride (39 g) was added with stirring and cooling on ice. The mixture was stirred at room temperature for 24 h. After dilution with ethyl ether, the mixture was washed successively with H_2O, diluted HCl, and aqueous NaCl. The organic layer was dried and evaporated. The residue was dissolved in dimethyl sulfoxide (150 ml), and NaCN (13 g) was added. The mixture was stirred at room temperature for 6 h. After dilution with hexane, the mixture was washed with H_2O. The organic layer was dried

and evaporated to give (*S*)-[2,2-$^2H_4$]-4-hydroxy-3-methylbutanenitrile THP ether as a pale yellow oil (19.1 g). To a stirred suspension of $LiAl^2H_4$ (4.85 g) in ethyl ether (200 ml), a solution of (*S*)-[2,2-$^2H_4$]-4-hydroxy-3-methylbutanenitrile THP ether (19.1 g) in ethyl ether (80 ml) was added dropwise at 0°C and the mixture was stirred at room temperature for 1 h and cooled on ice. To the mixture, 1 N NaOH (20 ml) and THF (20 ml) were added with stirring. The mixture was filtered through a Celite pad. The filtrate was dried and evaporated to give (*S*)-[3,3,4,4-$^2H_4$]-4-amino-2-methyl-1-butanol THP ether as a pale yellow oil (19.3 g): ^{1}H NMR 0.93 (3H × 1/2, d, $J = 6.8$ Hz), 0.94 (3H × 1/2, d, $J = 6.8$ Hz), 1.2–1.9 (9H, m), 3.13–3.27 (1H, m), 3.45–3.64 (2H, m), 3.86 (1H, m), 4.57 (1H, m).

*d_4-TAK-637 (**1**)*

The synthesis of d_4-TAK-637 was carried out using (*S*)-[3,3,4,4-$^2H_4$]-4-amino-2-methyl-1-butanol THP ether in place of (*R*)-4-amino-2-methyl-1-butanol THP ether according to a procedure similar to that described previously:[7] mp 227–229°C; ^{1}H NMR 0.91 (3H, d, $J = 7.0$ Hz), 2.08 (1H, m), 2.37 (3H, s), 2.97 (1H, dd, $J = 15, 1.4$ Hz), 3.45 (1H, dd, $J = 15, 11$ Hz), 3.99 (1H, d, $J = 15$ Hz), 5.46 (1H, d, $J = 15$ Hz), 6.83 (1H, dd, $J = 7.8, 1.8$ Hz), 7.05 (1H, d, $J = 7.8$ Hz), 7.26 (1H, d, $J = 7.8$ Hz), 7.34 (1H, dd, $J = 7.8, 1.8$ Hz), 7.47 (1H, dd, $J = 8.6, 4.4$ Hz), 7.48 (2H, s), 7.56 (1H, dd, $J = 8.6, 1.8$ Hz), 7.82 (1H, s), 8.91 (1H, dd, $J = 4.0, 2.2$ Hz). Analytically calculated for $C_{30}H_{21}D_4N_3O_2F_6$: C, 62.39; H, 3.66; D, 1.39; N, 7.28. Found: C, 62.41; H, 3.65; D, 1.35; N, 7.21. $[\alpha]_D$: +116.6° ($c = 0.541$, MeOH).

*d_4-Hydroxylated TAK-637 (**2**)*

Streptomyces subrutilus IFO13388 was inoculated to a 2-l Sakaguchi flask containing the medium (500 ml) and grown at 28°C for 48 h on a reciprocal shaker to obtain a seed culture. The seed culture was transferred to a 200-l fermentor containing 100 l of the same medium. The mixture was incubated at 28°C with aeration of 36 l/min and agitation of 100 rpm for 24 h. A solution containing 18 g of d_4-TAK-637 (20 mg/ml in dimethyl sulfoxide) was added to 100 l of the culture, and the mixture was incubated at 28°C for 24 h with the agitation. The reaction mixture was adjusted to pH 4 with 2 N H_2SO_4 and

extracted with ethyl acetate. The organic layer was concentrated under reduced pressure to afford a residue, which was purified by silica gel column chromatography. Elution with ethyl acetate–methanol (9 : 1) afforded d_4-hydroxylated TAK-637 as colorless crystals (5.97 g). Recrystallization from ethyl acetate–ethyl ether gave colorless crystals: mp 241–242°C; ^{1}H NMR 0.91 (3H, d, $J = 7.0$ Hz), 1.85 (1H, m), 2.09 (1H, m), 2.98 (1H, dd, $J = 15, 1.4$ Hz), 3.47 (1H, dd, $J = 15, 11$ Hz), 4.02 (1H, d, $J = 15$ Hz), 4.75 (2H, d, $J = 4.4$ Hz), 5.45 (1H, d, $J = 15$ Hz), 6.97 (1H, d, $J = 8.0$ Hz), 7.25 (1H, d, $J = 8.0$ Hz), 7.4–7.6 (6H, m), 7.81 (1H, s), 8.91 (1H, dd, $J = 4.0, 2.2$ Hz). Analytically calculated for $C_{30}H_{21}D_4N_3O_3F_6 \cdot 1/2H_2O$: C, 59.80; H, 3.68; D, 1.34; N, 6.97. Found: C, 59.76; H, 3.78; D, 1.40; N, 6.73. $[\alpha]_D$: $+94.2°$ ($c = 0.538$, MeOH).

Conclusion

We have demonstrated that a deuterium-labelled drug metabolite for use as an internal standard in an LC-MS assay can be prepared using microbial conversion with actinomycetes.

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