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Syntheses of (*R,S*)-naproxen and its 6-*O*-desmethylated metabolite labelled with ^{2}H

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Naproxen, a well-known non-steroidal anti-inflammatory drug, and its 6-*O*-desmethylated metabolite have been labelled with ^{2}H. (*R,S*)-Naproxen 7 labelled with ^{2}H was obtained in five steps using the commercially available [$^{2}H_3$]iodomethane 5 as the stable labelled reagent. The demethylation of 7 using 48% HBr in 1-butyl-3-methylimidazolium tetrafluoborate gave the corresponding ^{2}H-labelled 6-*O*-desmethylated metabolite 8.

Keywords: naproxen; metabolite; internal standard; deuterium; LC-MS

Introduction

Naproxen (namely (*S*)-2-(6-methoxynaphthalen-2-yl)propanoic acid) belongs to the non-steroidal anti-inflammatory class of therapeutic agents and is commonly used to reduce inflammation, pain and fever[1]. The compound is given orally and, before excretion almost exclusively in urine, is partially metabolized to its 6-*O*-desmethylated metabolite[2]. The determination of naproxen and its metabolites in biological fluids has been reported in the literature using different methods including liquid chromatography (LC) and LC-NMR[3]. More recently an analytical procedure has been described to profile the urinary metabolites of naproxen by LC-MS[4]. A renewed interest has been recently raised to develop a robust and validated LC-MS method to determine naproxen and its 6-*O*-desmethylated metabolite in biological fluids. Therefore, the preparation of the stable labelled versions of the title compounds was required for use as internal standards. In this paper the synthetic route to label naproxen and its 6-*O*-desmethylated metabolite is described.

Results and discussion

Many approaches can be followed to prepare suitable stable labelled versions of naproxen and its 6-*O*-desmethylated metabolite. In a recent paper, the synthesis of naproxen labelled with deuterium on the naphthyl ring, the methoxy group or both has been reported[5]. However, the described H-D exchange reaction gave mixtures with varying deuterium content that were not suitable for use as internal standards. The introduction of the isotopic label in the methoxy group, although convenient for the preparation of the stable labelled naproxen, is not applicable to its 6-*O*-desmethylated metabolite. A more attractive labelling site to obtain the two desired internal standards with a limited number of synthetic steps starting from the commercially available stable labelled iodomethane seemed to be the methyl group of the chiral side chain. Moreover, the use of the corresponding racemic mixtures was acceptable in this case with no need of the chiral separation step. The synthetic pathway followed is shown in Scheme 1. The commercially available 1-(6-methoxy-naphthalen-2-yl)-ethanone **1** was efficiently converted to the corresponding acid **3** via a Willgerodt–Kindler reaction in the presence of triethylbenzylammonium chloride (TEBA) as phase transfer catalyst[6]. After esterification of **3**, the obtained ethyl ester **4** was treated with sodium hydride in dry tetrahydrofuran then alkylated by adding a stoichiometric amount of [$^{2}H_3$]iodomethane **5**. The hydrolysis of the ester **6** in aqueous KOH gave, after purification by flash-chromatography, the desired deuterated (*R,S*)-naproxen **7** with a chemical purity of $>97\%$ and an overall chemical yield of 18% from **1**. The cleavage of the methyl ether group was achieved by refluxing **7** for about 12 h in 1-butyl-3-methylimidazolium tetrafluoborate ([Bmim][BF_4]) in the presence of 48% HBr[7]. After purification by flash-chromatography, the deuterated metabolite **8** was obtained $>97\%$ chemically pure. The yield of this step was about 30%. The above compounds were suitable for use as IS's for LC-MS determination of naproxen and its 6-*O*-desmethylated metabolite in biological fluids.

Experimental

Chemicals and materials: [$^{2}H_3$]Iodomethane (**5**; 99.5 at% ^{2}H) was purchased from Aldrich Chemical Co. All solvents and reagents were of analytical grade and were used without purification unless otherwise indicated. Isolute Flash Si columns (International Sorbent Technology) were used for flash-chromatography purifications.

Instrumentation and equipment: Chemical purities were determined by HPLC using a series-200 pump (Perkin-Elmer) equipped with series 200 solvent degasser (Perkin-Elmer), a

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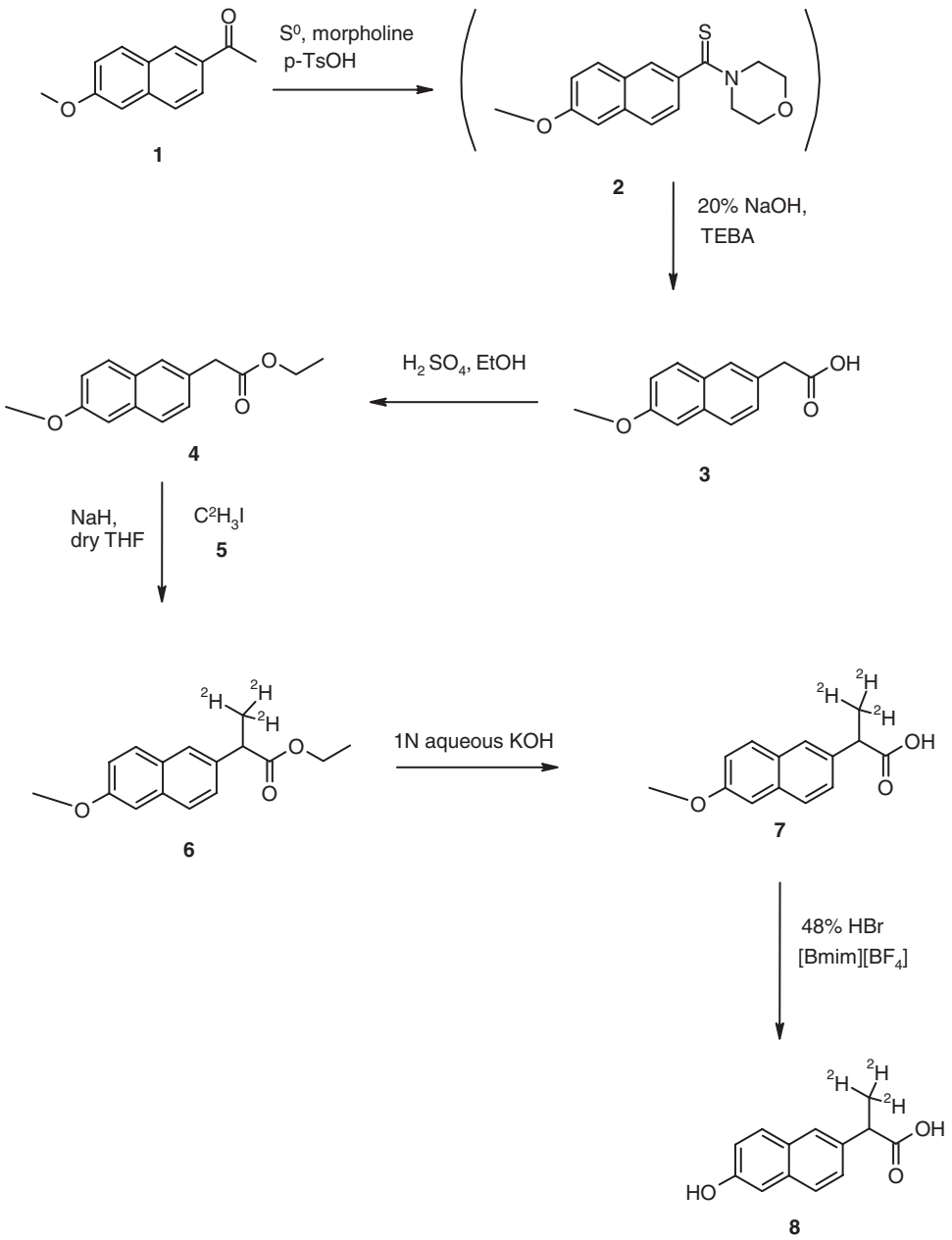

Scheme 1

series 200 auto-sampler (Perkin-Elmer) and a LC-235 UV diode array detector (Perkin-Elmer) connected with Turbochrom Client/Server (PENelson) as integrator via link 600 interface (Perkin-Elmer). NMR spectra were recorded on a 300 MHz spectrometer (Varian). MS spectra were acquired from an API 4000 mass spectrometer (Applied Biosystems).

Analytical methods: HPLC System: X-Terra Waters RP18 column (4.6 × 100 mm, 5 μm) eluting with H_2O:CH_3CN:TFA (trifluoroacetic acid) 90:10:0.1 by volume (A) and H_2O:CH_3CN:TFA 10:90:0.1 by volume (B): from 100% A to 0% A in 10 min; 5 min at 0% A; from 100% B to 100% A in 1 min; 4 min at 100% A. Flow rate: 1 ml/min. Column temperature: 25°C. Analytical wavelength: 255 nm.

(6-Methoxy-naphthalen-2-yl)-acetic acid (3)

1-(6-Methoxy-naphthalen-2-yl)-ethanone **1** (800 mg, 4 mmol), sulfur (256 mg, 8 mmol), *p*-toluenesulfonic acid monohydrate (26.6 mg, 0.14 mmol) and morpholine (2 ml, 24 mmol) were introduced into a reaction flask and stirred overnight at 125–130°C. After about 12 h the conversion to the intermediate **2** was completed (as determined by TLC on silica gel; *n*-hexane:ethyl acetate 8:2 by volume). The reaction mixture was cooled to room temperature and a solution of TEBA (46 mg, 0.2 mmol) in 20% NaOH (3 ml) was added. After stirring for additional 5 h at 100°C, the conversion to the desired intermediate **3** was observed (as determined by TLC on silica

gel; *n*-hexane:ethyl acetate 5:5 by volume). The mixture was cooled to room temperature and filtered. The filtrate was acidified to pH 2 with 6 M HCl, and the crude intermediate **3** (865 mg, 4 mmol) was obtained as a yellow solid that co-chromatographed with an authentic marker (TLC on silica gel; *n*-hexane:ethyl acetate 5:5 by volume; $R_f = 0.18$) and was used in the next step without purification.

(6-Methoxy-naphthalen-2-yl)-acetic acid ethyl ester (4)

A solution of **3** (865 mg, 4 mmol) in ethanol (15 ml) and concentrated H_2SO_4 (540 µl, 0.01 mmol) was stirred under reflux at 80°C for about 5 h. At the end of the reaction (as determined by TLC on silica gel; *n*-hexane:ethyl acetate 3:7 by volume) the mixture was diluted with water, transferred into a separating funnel and extracted with dichloromethane (3 × 5 ml). The organic phases were combined, washed with aqueous $NaHCO_3$ (50 ml) and dried over Na_2SO_4. After solvent evaporation to dryness, the obtained crude **4** was purified by flash-chromatography on a silica gel column using *n*-hexane then a mixture of *n*-hexane:ethyl acetate 9:1 by volume as eluting solvent systems. The collected fractions were combined as appropriate and after evaporation to dryness, the intermediate **4** (505 mg; 2.07 mmol) was obtained as a yellow-orange solid with an HPLC purity of 88% (see analytical methods; $R_t = 8.90$ min). The yield was approximately 52% from **1.**

(*R,S*)-2-(6-Methoxy-naphthalen-2-yl)-[3-$^2H_3$]propanoic acid ethyl ester (6)

A suspension of 60% NaH in mineral oil (84 mg; 2.01 mmol) in dry tetrahydrofuran (THF; 3.5 ml) was stirred at room temperature for 15 min then a solution of **4** obtained from the former reaction step (427 mg; 1.75 mmol; HPLC purity = 88%) in dry THF (2.5 ml) was added. After stirring for 1 h, [$^2H_3$] iodomethane (**5;** 109 µl; 1.75 mmol) in dry THF (10 ml) was introduced into the flask and the reaction mixture was stirred at room temperature overnight. The solvent was concentrated and the residue was taken up with water, transferred into a separating funnel and extracted with dichloromethane (3 × 5 ml). The organic phase was washed with brine (1 × 15 ml) and dried over Na_2SO_4. After solvent evaporation to dryness, crude **6** was obtained as a yellow solid and was purified by flash-chromatography on a silica gel column using mixtures of *n*-hexane:ethyl acetate from 9:1 up to 7:3 by volume as eluting solvent system. The collected fractions were combined as appropriate and after evaporation to dryness, **6** was obtained (160 mg; 0.61 mmol) as a white-yellow solid with an HPLC purity of 90% (see analytical methods; $R_t = 8.96$ min). The yield of this step was approximately 35%.

(*R,S*)-2-(6-Methoxy-naphthalen-2-yl)-[3-$^2H_3$]propanoic acid (7)

The intermediate **6** (160 mg; 0.61 mmol) and 1 N aqueous KOH (1 ml) were stirred at 70–75°C for 2 h. At the end of the reaction (as determined by HPLC; see analytical methods), the reaction mixture was acidified with 1 M HCl, diluted with water, transferred into a separating funnel and extracted with dichloromethane (3 × 5 ml). The organic phase was washed with brine (1 × 15 ml) and dried over Na_2SO_4. After solvent evaporation to dryness, the obtained crude **7** was purified by flash-chromatography on a silica gel column using dichloromethane followed by a mixture of dichloromethane:methanol 95:5 by volume as eluting solvent systems. The collected fractions were combined as appropriate and after solvent evaporation to dryness, compound **7** (127 mg; 0.54 mmol) was obtained as a white-yellow solid, >97% chemically pure (as determined by HPLC; see analytical methods; $R_t = 7.60$ min). The yield of this step was approximately 89%. MS (API-TurboIon-Spray-MS): *m/z* 234 ([MH]$^+$). ^{1}H NMR ($CDCl_3$; 300 MHz) δ 3.87(s, 1H, CH–CD_3); 3.92 (s, 3H, O–CH_3); 7.08–7.19 (m, 2H, naphthalene ring); 7.43 (dd, $J = 8.42$, 1.83 Hz, 1H, naphthalene ring); 7.69–7.75 (m, 3H, naphthalene ring).

(*R,S*)-2-(6-Hydroxy-naphthalen-2-yl)-[3-$^2H_3$]propanoic acid (8)

Compound **7** (40 mg; 0.17 mmol) and 48% HBr (200 µl; 1.71 mmol) in [Bmim][BF_4] (800 µl; 3.76 mmol) were stirred at reflux for about 12 h. At the end of the reaction (as determined by HPLC; see analytical methods) water and diethyl ether were added, the mixture was transferred into a separating funnel and the aqueous phase was extracted with diethyl ether (3 × 4 ml). The organic phases were combined, washed with brine (10 ml) and dried over Na_2SO_4. After solvent evaporation to dryness, the crude **8** was purified by flash-chromatography on a silica gel column using a mixture of dichloromethane:methanol 95:5 by volume as the eluting solvent system. The collected fractions were combined as appropriate and, after solvent evaporation to dryness, compound **8** (11.8 mg; 0.05 mmol) was obtained as a pink-brown solid, >97% chemically pure (as determined by HPLC; see analytical methods; $R_t = 6.55$ min).

The yield was approximately 30% from **7**. MS (API-TurboIon-Spray-MS): *m/z* 220 ([MH]$^+$). ^{1}H NMR ($CDCl_3$; 300 MHz) δ 3.87(s, 1H, CH–CD_3); 7.08–7.15 (m, 2H, naphthalene ring); 7.41 (dd, $J = 8.42$, 1.83 Hz, 1H, naphthalene ring); 7.68 (dd, $J = 17.4$, 8.61 Hz, 3H, naphthalene ring).

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