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Research Article

Fast and efficient tritium labelling of the nonsteroidal anti-inflammatory drugs naproxen, tolmetin, and zomepirac

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Summary

Fast and efficient tritium labelling of the nonsteroidal anti-inflammatory drugs naproxen, tolmetin and zomepirac is reported. Naproxen along with its (*R*)-enantiomer were labelled by catalytic tritium–halogen exchange of the corresponding 5-bromo derivatives providing [^{3}H]naproxen with a specific activity of 25.4 Ci/mmol. Tolmetin and zomepirac were labelled by the hydrogen isotope exchange reaction using Crabtree's catalyst. This provided [^{3}H]tolmetin and [^{3}H]zomepirac with specific activities of 80.8 and 64.3 Ci/mmol, respectively. All compounds were obtained in high radiochemical purity (> 98%). Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: tritium; tolmetin; zomepirac; naproxen; Crabtree's catalyst; NSAID

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are an important class of drugs with widespread applications. Members of this class include ibuprofen, naproxen, and tolmetin. They are used to reduce the pain and inflammation caused by many conditions such as osteoarthritis and rheumatoid arthritis as well as general pain relief (ibuprofen). Zomepirac, another member of the class, was voluntarily withdrawn from use due to adverse allergic reactions in 1983.[1]

Information on the metabolism of these compounds in man and animals and identification of the metabolic pathways can give important information on the cause of the adverse reactions this class of drugs gives rise to.[2]

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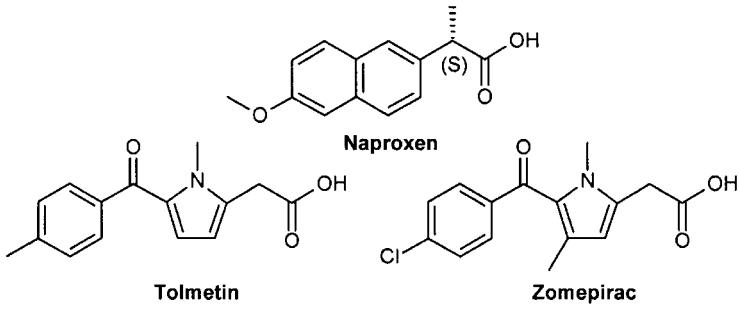


Figure 1. Structure of naproxen, tolmetin, and zomepirac

Labelling with radioactive isotopes such as tritium or carbon-14 is one of the most efficient and convenient tools for the labelling of biological compounds. In general, tritium labelling offers the advantage of short and fast synthetic routes compared to carbon-14. This, combined with the high specific radioactivity obtainable with tritium, makes tritium labelling very useful for exploratory *in vitro* and *in vivo* investigations.

Here, we report on the tritium labelling of naproxen, tolmentin, and zomepirac as well as the (*R*)-enantiomer of naproxen for use in *in vitro* and *in vivo* investigations (Figure 1).

Results and discussion

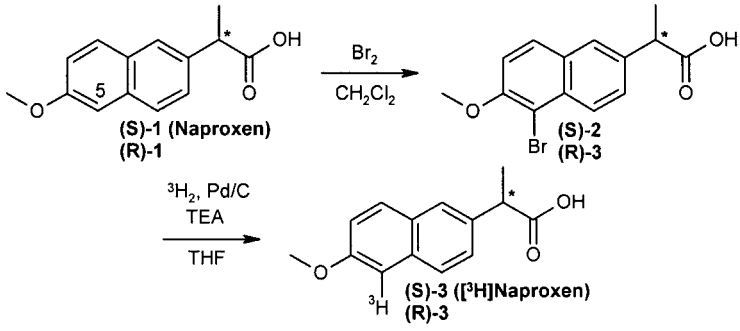
Carbon-14-labelled naproxen is commercially available with the labelling positioned in the *O*-methyl group. However, the *O*-methyl group is readily metabolized making this particular tracer unsuited for the intended use. Furthermore, we also wanted to label the (*R*)-enantiomer of naproxen, and therefore, we decided to look at an alternative labelling route.

One obvious way of introducing the tritium label would be by reduction of 2-(6-methoxy-2-naphthyl)propenoic acid. However, a number of issues arise with this synthetic strategy. First, although impressive enantioselectivities have been obtained in the asymmetric reduction of this compound using chiral ruthenium catalysts,[3,4] the reaction is highly dependant on the hydrogen pressure. In general, high pressures (> 100 atm) are needed in order to achieve high ee's,[4] which is incompatible with the use of tritium manifold equipment. Secondly, 2-(6-methoxy-2-naphthyl)propenoic acid is not commercially available, and while a number of procedures for the preparation of this compound have been published,[5] this makes a racemic approach (nonselective reduction with tritium gas followed by chiral separation) less appealing.

Previously, we have achieved good results with labelling of activated aromatics by introduction of bromine followed by tritium–halogen exchange.[6,7] If this could be applied to the present task, then, naproxen could be tritium-labelled in two simple steps, namely bromination and catalytic

tritium–halogen exchange. A further advantage of this approach would be the elimination of a chiral purification step, as the chiral center in naproxen is not affected in this synthetic sequence. This approach would also be easily applicable to the labelling of the (*R*)-enantiomer, as both enantiomers are commercially available.

Treatment of naproxen ((***S*)-1**) with an excess of bromine in dichloromethane gave the crystalline bromo compound **(*S*)-2** in quantitative yield (Scheme 1). Bromination was expected to take place in the 5-position analogous to the bromination of 2-naphthol[8] and this was confirmed by 2D ^{1}H-NMR (HMBC). Subsequent catalytic dehalogenation using tritium gas and Pd/C in THF proceeded smoothly to give crude **(*S*)-3**, which was purified by RP HPLC to give [^{3}H]naproxen ((***S*)-3**) in 70% overall yield with a radiochemical purity >98%. The specific activity was determined to be 25.4 Ci/mmol as determined by mass spectroscopy.



Scheme 1. Tritium labelling of naproxen and the (*R*)-enantiomer of naproxen

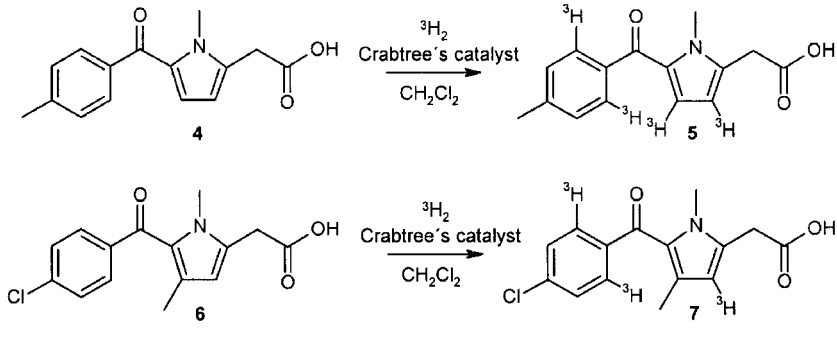
Then, the (*R*)-enantiomer of naproxen ((***R*)-1**) was tritium-labelled in exactly the same manner. Conversion into the bromo compound **(*R*)-2** was performed in quantitative yield followed by catalytic dehalogenation giving **(*R*)-3** in 60% overall yield with a radiochemical purity >98% and a specific activity of 25.8 Ci/mmol.

Tolmetin[9] and zomepirac[10] have been labelled with carbon-14 as part of the development of these drugs. Both compounds were labelled in the ketone carbonyl group, however, no information on the labelling routes are available. Likewise, no information on the labelling position or route is available for the reported tritium labelling of tolmetin.[11] Deuterium[12]- and tritium[13]-labelled zomepirac have also been reported. Deuterium-labelled zomepirac was synthesized in five steps with the label being introduced from D_2O. Tritium labelling using this method would be feasible; however, it did not seem appealing due to the multi-step sequence and the practical issues concerning use of tritium-labelled water. Tritium-labelled zomepirac was synthesized by alkylation of *N*-desmethyl zomepirac with tritium-labelled methyl iodide.

This is a short and practical route; however it still requires the synthesis of the *N*-desmethyl precursor.

A popular alternative to tritium labelling via multi-step synthetic routes is the use of the iridium-catalysed hydrogen isotope exchange reaction. A popular choice of catalyst for this reaction is Crabtree's catalyst ([Ir(cod)(Cy_3P)(Py)]PF_6) due to its stability and commercial availability. This reaction has the benefit of working directly on the target compound, thus negating the need for preparation of a precursor. Furthermore, the position of labelling is generally very predictable with the labelling taking place predominantly in the *ortho*-position(s) of aromatic systems possessing suitable directing groups such as a carbonyl.[14]

We considered both tolmetin and zomepirac to be ideal candidates for the hydrogen isotope exchange reaction, as the keto and acid carbonyl moieties should be able to direct the labelling. Thus, treatment of tolmetin (**4**) with tritium gas and one equivalent of Crabtree's catalyst in dichloromethane proceeded smoothly to give crude **5**, which was purified by RP HPLC to give [^{3}H]tolmetin (**5**) in 28% yield with a radiochemical purity >98% (Scheme 2). Likewise, zomepirac (**6**) was labelled using the same procedure to give [^{3}H]zomepirac (**7**) in 22% yield with a radiochemical purity >98%. The specific activities were determined to be 80.8 and 64.3 Ci/mmol for **5** and **7**, respectively.



Scheme 2. Tritium labelling of tolmetin and zomepirac

The labelling was determined by tritium NMR to have taken place in the two *ortho*-positions in the benzene moiety as well as the available positions in the pyrrole moiety (two for **5** and one for **7**) as shown in Scheme 2. Thus, the labelling had been influenced by both directing groups, and is consistent with the proposed 5- and 6-membered transition states.[15–17]

Conclusion

In summary, we have developed fast and efficient synthetic routes for tritium labelling of the nonsteroidal anti-inflammatory drugs naproxen, tolmetin, and

zomepirac with high specific activities. Naproxen and its (*R*)-enantiomer were labelled with tritium in two steps by catalytic tritium–halogen exchange of the corresponding 5-bromo derivatives. This provided [^{3}H]naproxen (25.4 Ci/mmol) as well as the (*R*)-enantiomer (25.8 Ci/mmol) in 70 and 60% overall yield, respectively. Tolmetin and zomepirac were labelled in one step by the hydrogen isotope exchange reaction using Crabtree's catalyst. This provided [^{3}H]tolmetin (80.8 Ci/mmol) and [^{3}H]zomepirac (64.3 Ci/mmol) in 28 and 22% overall yield, respectively. In all cases the labelled compounds were obtained in high radiochemical purities. The cases presented highlight two of the fastest and most efficient methods for tritium labelling, namely halogenation of activated aromatic systems followed by catalytic tritium–halogen exchange and the iridium-catalysed hydrogen isotope exchange reaction.

Experimental

General

Reactions using tritium gas were performed on a custom built tritium handling unit from RC Tritec AG, Switzerland, who also supplied the tritium gas. Tritium gas was stored in the form of U^3H_3 and was prepared *in situ* by heating the uranium bed. Tolmetin, sodium salt dihydrate and zomepirac, sodium salt were supplied by Sigma, naproxen by Riedel-de Haën, the (*R*)-enantiomer of naproxen by The Pharmaceutical University of Denmark and Crabtree's catalyst ([Ir(cod)(Cy_3P)(Py)]PF_6) by Aldrich. The free acids of tolmetin and zomepirac were prepared by extraction with ethyl acetate from an aqueous HCl solution of the corresponding salts. All other reagents and solvents were of analytical grade and used without further purification. HPLC was performed using a Merck Hitachi Intelligent Pump L6200A equipped with a Supertherm Column Thermostat (Mikrolab Aarhus) (set at 40°C), a Merck LC Organizer with a Rheodyne injector, and a Merck Hitachi UV Detector L4000 (detection at 248 nm for tolmetin/zomepirac and 276 nm for naproxen). Detection of tritium was performed on a Canbarra Packard flow detector 500TR. Analytical and preparative HPLC was performed on a RP C18 column (4.6 × 250 mm, 5 μm, OdDMeSi 120 Å, Novo Nordisk) with a flow of 1.0 ml/min. using one of the two following systems. System 1: 75% A 0–30 min followed by 0% A for 10 min; and system 2: 65% A 0–30 min followed by 0% A for 10 min (A: 10% acetonitrile in 0.1% aq. TFA; B: 90% acetonitrile in 0.1% aq. TFA). Quantitative radioactivity measurements were performed on a Packard Tri-Carb 1000 liquid scintillation analyzer using Ultima Flo™ M (PerkinElmer) as liquid scintillation cocktail. Specific activities were determined on a Sciex API 300 mass spectrometer equipped with an ionspray interface. ^{1}H- and ^{3}H-NMR spectra were recorded on a Bruker DRX400 spectrometer.

(2S)-2-(5-Bromo-6-methoxy-2-naphthyl)propanoic acid (**(*S*)-2**)

Bromine (263 mg, 1.64 mmol) in dichloromethane (1.2 ml) was added to a solution of (*2S*)-2-(6-methoxy-2-naphthyl)propanoic acid (**(*S*)-1**) (76.7 mg, 0.333 mmol) in dichloromethane (15 ml). The reaction mixture was stirred for 1 h at room temperature and then concentrated to provide **(*S*)-2** as colourless crystals (104 mg, quantitative).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.61 (3 H, d, $J = 7.1$), 3.92 (1 H, q, $J = 7.1$), 4.03 (3 H, s), 7.28 (1 H, d, $J = 9.6$, partly hidden by the $CDCl_3$ peak), 7.54 (1 H, dd, $J = 2.0, 9.1$), 7.71 (1 H, d, $J = 1.5$), 7.79 (1 H, d, $J = 9.0$), 8.19 (1 H, d, $J = 9.1$).

(2S)-2-(5[^{3}H]-6-Methoxy-2-naphthyl)propanoic acid ([^{3}H]naproxen) (**(*S*)-3**)

Pd/C (10%, 3.98 mg) and Et_3N (10 µl, 0.072 mmol) were added to a solution of (*2S*)-2-(5-bromo-6-methoxy-2-naphthyl)propanoic acid (**(*S*)-2**) (3.17 mg, 0.010 mmol) in THF (0.4 ml). The reaction mixture was degassed by three freeze/thaw cycles and stirred overnight with tritium gas (8.7 Ci). Excess tritium gas was reabsorbed on to a uranium waste bed followed by filtration of the reaction mixture and lyophilization with ethanol (3 × 1 ml) in order to remove labile tritium. Finally, the residue was dissolved in ethanol (5 ml) to give crude **(*S*)-3** (238 mCi). Approximately 16.6 mCi of this solution was purified by HPLC (System 2). This provided **(*S*)-3** (12.34 mCi, 70% based on **(*S*)-2** with a radiochemical purity > 98% (System 2). The specific radioactivity was determined to be 25.4 Ci/mmol by MS.

(2R)-2-(5-Bromo-6-methoxy-2-naphthyl)propanoic acid (**(*R*)-2**)

(*2R*)-2-(6-Methoxy-2-naphthyl)propanoic acid (**(*R*)-1**) (43.7 mg, 0.190 mmol) was reacted according to the procedure for **(*S*)-2** to give **(*R*)-2** as colourless crystals (60.0 mg, quantitative). ^{1}H-NMR as for compound **(*S*)-2**.

(2R)-2-(5[^{3}H]-6-Methoxy-2-naphthyl)propanoic acid (**(*R*)-3**)

(*2R*)-2-(5-Bromo-6-methoxy-2-naphthyl)propanoic acid (**(*R*)-2**) (3.64 mg, 0.012 mmol) was tritiated according to the procedure for **(*S*)-2** to give crude **(*R*)-3** (317 mCi). Approximately 22.2 mCi of the crude product was purified by HPLC (System 2). This provided **(*R*)-3** (12.94 mCi, 60% based on **(*R*)-2** with a radiochemical purity > 98% (System 2). The specific radioactivity was determined to be 25.8 Ci/mmol by MS.

[^{3}H]1-Methyl-5-(p-toluoyl)pyrrole-2-acetic acid ([^{3}H]tolmetin) (**5**)

Crabtree's catalyst ([Ir(cod)(Cy_3P)(Py)]PF_6) (11.0 mg, 0.014 mmol) and 1-methyl-5-(p-toluoyl)pyrrole-2-acetic acid (tolmetin) (**4**) (3.6 mg, 0.014 mmol) were dissolved in dichloromethane (0.4 ml). The reaction mixture was degassed

by three freeze/thaw cycles and stirred overnight with tritium gas (8.5 Ci). Excess tritium gas was reabsorbed on to a uranium waste bed followed by filtration of the reaction mixture and lyophilization with ethanol (3×1 ml) in order to remove labile tritium. Finally, the residue was dissolved in ethanol (5 ml) to give crude **5** (1836 mCi). Approximately 46 mCi of this solution was purified by HPLC (System 1). This provided **5** (7.95 mCi, 28% based on **4**) with a radiochemical purity >98% (System 1). The specific radioactivity was determined to be 80.8 Ci/mmol by MS.

[^{3}H]5-(p-Chlorobenzoyl)-1,4-dimethylpyrrole-2-acetic acid ([^{3}H]zomepirac) (**7**)

5-(*p*-Chlorobenzoyl)-1,4-dimethylpyrrole-2-acetic acid (zomepirac) (**6**) (2.1 mg, 0.007 mmol) was tritiated according to the procedure for **5** to give crude **7** (1609 mCi). Approximately 96.5 mCi of the crude product was purified by HPLC (System 2) to provide **7** (6.02 mCi, 22% based on **6**) with a radiochemical purity >98% (System 2). The specific radioactivity was determined to be 64.3 Ci/mmol by MS.

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