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Synthesis of Tritium-Labelled Metabolites of Ibuprofen

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Summary

The two major metabolites of ibuprofen, 2-[4-(2-carboxypropyl)phenyl]propionic acid and 2-[4-(2-methyl-2-hydroxypropyl)phenyl]propionic acid, were labelled with tritium. The hydroxy-metabolite containing tritium at α- and β-carbons of the propionic acid moiety was prepared by catalytic reduction of the olefinic double bond in 2-[4-(2-methyl-2-hydroxypropyl)phenyl]propenoic acid with tritium-enriched hydrogen. Tritium-labelling at the two carbon atoms α to the two carboxyl groups of the carboxy-metabolite was achieved by initially exchanging the carboxyl hydrogens of the corresponding dimalonic acid derivative synthesised in this work, followed by its decarboxylation. In this process one of the carboxyl groups of each of the malonic acid components is lost as carbon dioxide, transferring its tritium/hydrogen atom to the α-carbon.

Keywords: Ibuprofen metabolites, tritium, decarboxylation and catalytic reduction

Introduction

Ibuprofen has been extensively studied since its introduction into clinical practice about three decades ago. Epidemiological studies (1, 2 and 3) and experiments with diabetic rats (4) have shown that ibuprofen reduces the risk of cataract, although its mode of action is unknown.

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However, it is possible that ibuprofen may bring about its biological effect, *vis-a-vis* cataract, through the action of its metabolites (5). In an attempt to shed light on the mechanism of protection against cataract by ibuprofen, we set out to synthesise and radiolabel its metabolites to be subsequently used for protein binding studies, and to study their effects on lens enzymes and lens proteins, etc. The synthesis of the hydroxy-metabolite of ibuprofen (2) has been reported, whereas that of the carboxy-metabolite (8) has not, to our knowledge. Hence, in this paper, we describe a synthetic route for the preparation of the carboxy-metabolite from commercially-available starting materials, in addition to procedures for the convenient radiolabelling of both the hydroxy- and the carboxy-metabolites.

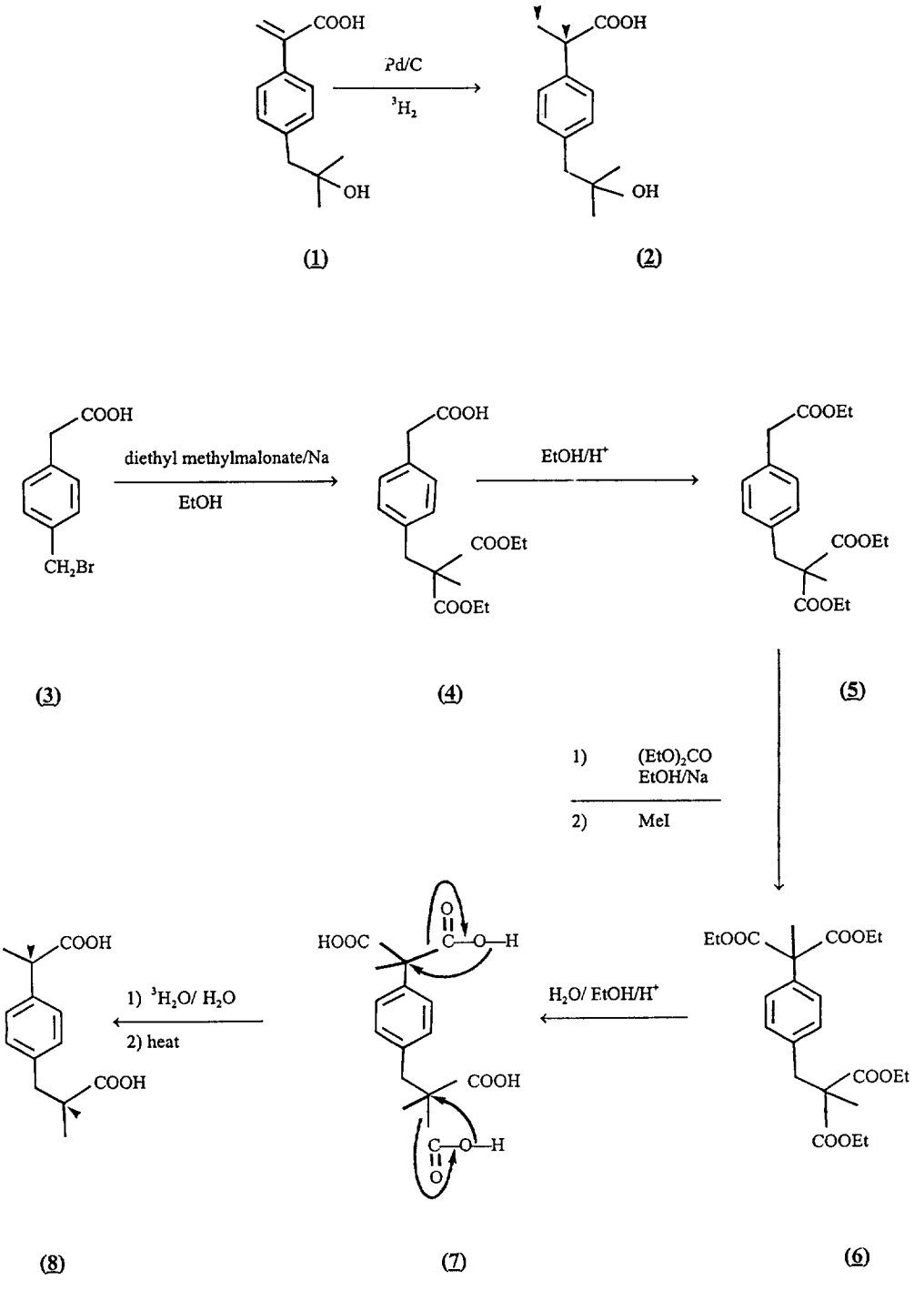
Experimental

Reagents of analytical grade were used. Preparative silica gel (70-230 mesh, 60 A°) and analytical silica plates of thickness 0.25 mm were from Aldrich Chemical Co (Gillingham, UK). Tritiated water was purchased from Amersham International (Amersham, UK). Ethanol was dried by heating under reflux, followed by distillation, over magnesium turnings. Tetrahydrofuran (THF) was dried by stirring with sodium shavings overnight and subsequent distillation. The products were extracted, where it mentions so, with ethyl acetate at least twice and the combined extracts were washed with water, dried with anhydrous magnesium sulphate and the solvent was evaporated off under reduced pressure. Radioactivity was determined with a Wallac 1211 Rackbeta liquid scintillation counter in Scintran Cocktail T (BDH Chemicals, Poole, UK).

[2,3-^{3}H]-2-[4-(2-methyl-2-hydroxypropyl)phenyl]propionic acid (2)

The precursor, 2-[4-(2-methyl-2-hydroxypropyl)phenyl]propenoic acid (1), of the title compound was prepared by the procedure described by Kurtz and Houser (6). The olefinic double bond of this compound was saturated by an adaptation of the procedure given in reference (6), as follows.

To a solution of (1) (220 mg, 1.08 mmol) in dry THF (20 ml) was added 10% Pd/C (60 mg) and the mixture was magnetically stirred. Tritium/hydrogen gas was generated by allowing tritiated water



Synthesis of the major metabolites of ibuprofen (2) and (8). ▼ denotes the position of tritium.

(2 µl, 5.4 mCi) in dry ethanol (50 µl) to react with sodium (100 mg) in dry THF (10 ml). The generated gas was fed into a closed circuit consisting of the reaction vessel containing (1) and the catalyst in THF, a peristaltic pump, and a balloon to buffer the changes in the pressure of the gas. The function of the pump was to bubble the recycled tritium/hydrogen gas into the reaction medium. Reduction took place overnight. The extent of the progress of the reaction was checked by the diminution of the uv absorption at 255 nm of the solution. The remaining unsaturation was reduced by bubbling hydrogen through the solution for 1 hour. The solution was passed through a small column of Celite to filter off the catalyst, evaporated to dryness *in vacuo* and the product (formed quantitatively) was recrystallised from ethyl acetate/hexane twice, giving (2) (135 mg; specific radioactivity: 5.1 mCi $mmol^{-1}$; Rf : 0.42, ethyl acetate-hexane 3 : 2; mp: 124-125.5°, Lit (6): 120-122°). Its ^1H nmr spectrum had δ ($CDCl_3$, 200 MHz) 7.28 (d, 2H), 7.18 (d, 2H), 6.5-5.7 (broad, deuterium-exchangeable, 2H), 3.72 (q, 1H), 2.73 (s, 2H), 1.50 (d, 3H) and 1.25 (s, 6H).

4-(2,2-dicarboethoxypropyl)phenylacetic acid (4)

4-(Bromomethyl)phenylacetic acid (3) (5.0 g, 21.8 mmol) was dissolved in dry ethanol (100 ml) to which was added, at room temperature, a solution of diethyl methylmalonate (15 g, 86 mmol) and sodium (1.7 g, 74 mmol) in ethanol (80 ml). After 1/2 hour, 5% aqueous hydrochloric acid (300 ml) was added and the solution was extracted with ethyl acetate, as described earlier, and the solvent was removed *in vacuo*. The residue was recrystallised from hexane, giving the product (4) (4.8 g, 14.9 mmol; yield: 68.3%; Rf : 0.40, hexane/ethyl acetate 7 : 4; mp: 77-78°). Its ^1H nmr spectrum revealed δ ($CDCl_3$, 200 MHz) 9.5-8.5 (broad, deuterium-exchangeable), 7.18 (d, 2H), 7.12 (d, 2H), 4.20 (q, 4H), 3.61 (s, 2H), 3.21 (s, 1H), 1.34 (s, 3H) and 1.26 (t, 6H).

Diethyl 2-[4-(2,2-dicarboethoxypropyl)phenyl]-2-methylmalonate (6)

The free carboxyl group of (4). (4.8 g, 14.9 mmol). was esterified with ethanol catalysed by concentrated sulphuric acid in the usual manner. The triethyl ester was worked up as described

earlier to give (5) (Rf : 0.51, hexane/ethyl acetate 4 : 1) which was then dissolved in diethyl carbonate (100 ml) and heated up to about 100°. Sodium (0.5 g, 22 mmol) in ethanol (50 ml) was added over 20 min at such a rate that ethanol gently distilled over through a column packed with broken glass. Heating was continued for a further 1/2 hour, after which time there was no more ethanol distilling over. The solution was allowed to cool to room temperature. Subsequently, a large excess of methyl iodide (15 ml) was added to the solution which was allowed to stand at room temperature for 1 hour. Water (50 ml) was then added and the water-immiscible layer was separated, washed with water (50 ml) and dried with magnesium sulphate. The solvent was removed *in vacuo*. The liquid residue was applied to a silica gel chromatography column (3 X 32 cm) and eluted with up to 10% ethyl acetate in hexane. The second compound eluted was collected and the solvent was evaporated off *in vacuo*, yielding (6) (5.3 g, 12.1 mmol; yield : 81.2%; Rf : 0.48, hexane/ethyl acetate 4 : 1). Its ^{1}H nmr spectrum revealed δ ($CDCl_3$, 200 MHz) 7.23 (d, 2H), 7.08 (d, 2H), 4.28-4.05 (m, 8H), 3.20 (s, 2H), 1.82 (s, 3H), 1.32 (s, 3H) and 1.26 (t, 12H).

[2-^{3}H]-2-[4-(2-carboxy-[2-^{3}H]propyl)phenyl]propionic acid (8)

The tetraethyl ester (6) (5.3 g, 12.1 mmol) was hydrolysed by heating under reflux for 1 hour in a mixture of water (120 ml), ethanol (50 ml) and sodium hydroxide (20 g). The product was isolated, after acidification with dilute hydrochloric acid, by extraction with ethyl acetate, giving (7) (3.9 g, 12.0 mmol). Its ^{1}H nmr spectrum had δ (D_2O, 200 MHz) 6.98 (d, 2H), 6.82 (d, 2H), 2.79 (s, 2H), 1.46 (s, 3H) and 0.96 (s, 3H).

The tetracarboxylic acid (7) (124 mg, 0.38 mmol) was dissolved in dry THF (4 ml) to which was added tritiated water (10 µl, 27 mCi) and the solution was stirred overnight to replace some of the carboxyl hydrogens with tritium. The solvent was evaporated off *in vacuo* and the residue was heated in an oil bath at about 180° until evolution of gas stopped (about 10 min). The residue was dissolved in ethanol (5 ml) and evaporated to dryness *in vacuo*. This operation was repeated several times to remove all the exchangeable tritium. The product was purified by preparative thin layer

chromatography (hexane/ethyl acetate 1 : 1), giving (8) (53 mg; 2.2 mCi; chemical yield : 58.7%) to which was added similarly prepared unlabelled (8) (170 mg). The combined solid was recrystallised from hexane/ethyl acetate three times, affording (8) (126 mg; 2.45 mCi $mmol^{-1}$; Rf: 0.41, ethyl acetate/hexane 3 : 2; mp : 119-121.5°). Its ^1H nmr spectrum showed δ ($CDCl_3$, 200 MHz; * indicates fine "splittings") 11.80-11.20 (broad, deuterium-exchangeable), 7.27 (d, 2H), 7.18 (d, 2H), 3.85-3.62 (m*, 1H), 3.15-2.95 (m*, 1H), 2.85-2.55 (m*, 2H), 1.52 (d*, 6H), 1.17 (d*, 6H); and its ^{13}C nmr spectrum had (the results of DEPT analysis showing the type of carbon are given in brackets) δ 182.98, 181.41, 138.42, 138.34, 137.99, 137.91, 129.45 (CH), 127.76 (CH), 44.88 (CH), 41.20 (CH), 40.92 (CH), 38.62 (CH_2), 17.91 (CH_3), 17.73 (CH_3), 16.81 (CH_3) and 16.58 (CH_3).

Results and discussion

The catalytic reduction of (1) and the decarboxylation of (7) were first explored by using unlabelled water. Having optimised the conditions, tritiated water was employed to prepare the labelled compounds. The identity and purity of the final products and the intermediates were established by ^1H nmr spectroscopy and thin layer chromatography, and melting point determinations, where appropriate.

The reduction of (1) was carried out at atmospheric pressure, in contrast to a higher pressure (approx 3.5 atm) previously reported (6). In order to reduce to a minimum the amount of the unreacted tritium released into the atmosphere, in the reaction leading to (2), the tritiated water was used in a smaller quantity than actually required to saturate the double bonds completely. Then, complete saturation was achieved by bubbling hydrogen through the solution.

The reaction leading to the tetraethyl ester (6) is reversible and hence ethanol which is also one of the products had to be removed to drive the reaction to completion (7). After allowing the solution to cool to room temperature, the sodio-derivative of the tetraester was methylated, using methyl iodide, without the prior isolation of the tetraester. This gave (6).

Some of the carboxyl protons of the acid (7) were exchanged for tritium. On heating (7), one of the two carboxyl groups of each of the two malonic acid moieties is lost in the form of carbon dioxide, transferring its tritium/hydrogen to the α-carbons.

Fine "splittings" of some of the ^{1}H nmr peaks were observed as a result of the presence of two diastereomers of (8), since it has two asymmetric centres. This is also reflected by some closely situated pairs of peaks in the ^{13}C nmr spectrum.

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References

1. van Heyningen R. and Harding J. J. — Lancet i, 111(1986)

2. Harding J. J. — Cataract: Biochemistry, Pharmacology and Epidemiology; Chapman and Hall, London, 1991.

3. Harding J. J., Egerton M. and Harding R. S. — Acta Ophthalmol. 67: 518 (1989)

4. Blakytny R. and Harding J. J.— Exp. Eye Res. 54: 509 (1992)

5. Shyadehi A. Z. and Harding J. J. — Biochem. Pharmacol. 42: 2077 (1991)

6. Kurtz R. R. and Houser D. J. — J. Org. Chem. 46: 202 (1981)

7. Wallingford V. H., Homeyer A. H. and Jones D. M. — J. Am. Chem. Soc. 63: 2056 (1941)

