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# Synthesis, Characterization, and Activity of Metabolites Derived from the Cyclooxygenase-2 Inhibitor Rofecoxib (MK-0966, Vioxx™)

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**Abstract**—Metabolites of the COX-2 inhibitor rofecoxib (MK-0966, Vioxx™) were prepared by synthetic or biosynthetic methods. Metabolites include products of oxidation, glucuronidation, reduction and hydrolytic ring opening. Based on an in vitro whole blood assay, none of the known human metabolites of rofecoxib inhibits COX-1 nor contributes significantly to the inhibition of COX-2. © 2000 Elsevier Science Ltd. All rights reserved.

## Introduction

Rofecoxib (4-[4-methylsulphonylphenyl]-3-phenylfuran-2(5*H*)-one, **1**) is a potent and selective COX-2 inhibitor that has been shown to be effective for the treatment of the symptoms of osteoarthritis and the relief of acute pain.[1,2] Rofecoxib is efficacious in models of pain and inflammation with a potency comparable to indomethacin but with considerably improved gastrointestinal tolerability as compared to nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit both COX-1 and COX-2.[3–5] In the case of rofecoxib, a 37- to 75-fold selectivity ratio of inhibition of COX-2 over COX-1 has been reported using in vitro whole blood assays.[3,6] Furthermore, no inhibition of COX-1 has been observed in clinical trials as measured by serum thromboxane $B_2$ production, even at doses exceeding the maximal analgesic effective dose.[7]

Drugs are often excreted as metabolites that result from enzymatic modifications such as oxidation, reduction, or conjugate formation. These metabolites may circulate in the blood stream and may be distributed to the site of pharmacological activity. Therefore, it is important to know if any potential circulating metabolites of rofecoxib have COX-2 inhibitory activity. Furthermore, the metabolites may be excreted through renal or biliary routes and, therefore, metabolites may be present in significant levels in the kidney, liver, and intestine. Because the inhibition of COX-1 has been associated with the gastrointestinal side effects of NSAIDs,[1,5] it was important to determine whether metabolites of rofecoxib could be inhibitors of COX-1. This paper outlines the syntheses of eight compounds that were observed as metabolites in human[8] and animal[9] studies. The pharmacological activities of these metabolites, as measured by human whole blood COX-1 and COX-2 assays, are reported.

## Results and Discussion

Initial in vitro biotransformation experiments were carried out with rat hepatic microsomal incubations supplemented with NADPH and with rat hepatocytes under standard conditions.[10,11] Analysis of the mixtures, as described below, indicated that oxidation of the 5-position to yield 5-hydroxy rofecoxib **2** and glucuronidation of this hydroxy metabolite to yield the diastereomeric β-*O*-glucuronides **3** constituted important pathways in vitro in the rat (Scheme 1). HPLC analyses of the incubates were performed under reverse-phase

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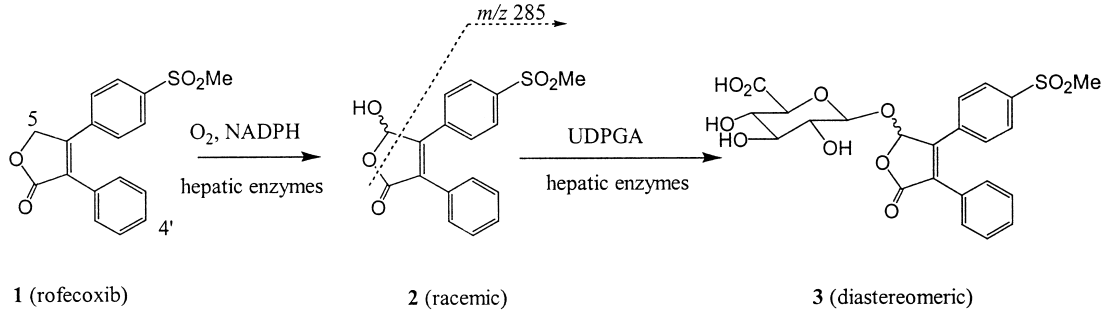
conditions, and the metabolite retention times are reported in Table 1. This pathway was also observed in rat in vivo studies.[9]

Characterization of the incubates by capillary HPLC-LSIMS on a JEOL-HX110 mass spectrometer operated in positive ion mode[12] indicated that **2** was an oxidation product of rofecoxib because the observed $(M+H)^+$ ion at 331 daltons was 16 mass units higher than for rofecoxib (*m*/*z* 315). Furthermore, the racemic 5-hydroxy metabolite (**2**) was proposed because fragmentation observed in negative ion mode yielded an ion with *m*/*z* 285, as shown in Scheme 1. The mass spectrum of the 5-*O*-glucuronides (**3**) in positive ion mode gave $(M+NH_4)^+$ ions at *m*/*z* 524, consistent with addition of oxygen and glucuronic acid ($-H_2O$).

The synthetic 5-hydroxy material (**2**) was obtained through air oxidation of rofecoxib. This was achieved by stirring a solution of rofecoxib in a flask opened to air in the presence of charcoal (Scheme 2). The synthetic standard (**2**) gave the same chromatographic and mass spectral data as the biosynthetic metabolite (**2**). The 5-*O*-glucuronides (**3**) were prepared from 5-hydroxy rofecoxib (**2**) using rat hepatic microsomal incubations fortified with uridinediphospho-glucuronic acid.[10] A 40 mL incubation of a 200 μM solution of 5-hydroxy rofecoxib (2.6 mg) generated a 70% yield of a mixture of the two diastereomeric glucuronides after 2.25 h in a ratio of approximately 1:24, as indicated by reverse phase HPLC. The products were isolated using a combination of solid-phase extraction and preparative HPLC similar to that reported previously.[11] Specifically, preparative HPLC was accomplished on a YMC ODS-AM column (20×50 mm) with 10 mL/min mobile-phase flow rate. The mobile phase consisted of methanol and 20 mM ammonium acetate in a gradient, with methanol increasing from 10 to 90% over 20 min. Electrospray mass spectrometric detection on a Finnigan SSQ mass spectrometer was used to automate peak detection and collection. $^1$H NMR of the isolated materials at 500 MHz showed characteristic resonances for the anomeric protons at ~4.7 ppm. The coupling constants were 7.9 Hz, consistent with *trans*-diaxial coupling as expected for the formation of β-linked *O*-glucuronides.[11]

In vivo experiments following dosing of rofecoxib in animal models and humans indicated that both metabolites **2** and **3** were present in urine and bile samples, however, the *O*-glucuronides **3** were more abundant than the 5-hydroxy lactone **2**.[8,9]

Following dosing of $^{14}$C-labeled rofecoxib in the dog, a polar urinary metabolite was isolated by a combination of reverse-phase and normal-phase HPLC. $^1$H NMR and mass spectral analysis of this metabolite suggested that it was a hydroxybutanoic acid metabolite of rofecoxib (accurate mass *m*/*z* 335.0954, theoretical *m*/*z* 335.0953) corresponding to reduction of the double bond and hydrolysis of the lactone. The two stereoisomeric hydroxybutanoic acids (*erythro* and *threo*) and their corresponding *cis* and *trans* lactone analogues (**4**–**7**) could not be generated biosynthetically from




**Scheme 1.** In vitro metabolites of rofecoxib in rat.

**Table 1.** Retention times and human whole blood assay $IC_{50}$ values of rofecoxib and the synthetic metabolites

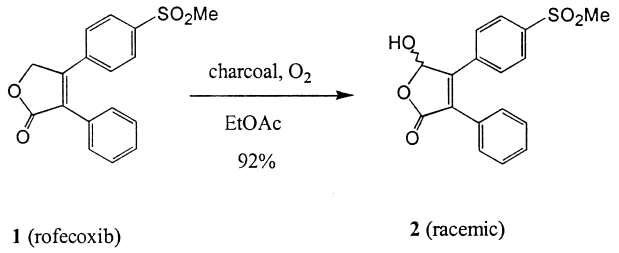
| Compound | Retention[a] time (min) | COX-1[b] | | COX-2[b] | |
|---|---|---|---|---|---|
| | | $IC_{50}$ (μM) | N (donor) | $IC_{50}$ (μM) | N (donor) |
| Rofecoxib (**1**) | 17.0 | 18.8±0.9 | 211 | 0.53±0.02 | 614 |
| 5-Hydroxy (**2**) | 16.0 | >100 | 3 | 8.7±2.9 | 3 |
| 5-*O*-Glucuronides (**3**) | 12.4/13.7 | >100 | 6 | >33 | 3 |
| *erythro*-Hydroxybutanoic acid (**4**) | 5.6 | >100 | 3 | >33 | 3 |
| *cis*-Lactone (**5**) | 14.0 | >100 | 3 | >100 | 3 |
| *trans*-Lactone (**6**) | 15.3 | >100 | 3 | >30 | 3 |
| *threo*-Hydroxybutanoic acid (**7**) | 7.7 | >100 | 3 | >33 | 3 |
| 4′-Hydroxy (**8**) | 14.0 | 6.5±1.5 | 6 | 4.8±1.3 | 6 |
| 4′-*O*-Glucuronide (**9**) | 8.4 | >100 | 6 | >33 | 6 |

[a]HPLC analysis was performed on a Zorbax-C18 column (4.6 mm×15 cm) with UV detection at 245 nm. The mobile phase flow rate was 1 mL/min with a gradient for which solvent A was 20 mM ammonium acetate and B was methanol. The methanol was increased from 15 to 85% over 25 min.
[b]Whole blood assays were performed as described in ref 3.

rofecoxib in rat microsomal or hepatocyte[11] incubations. These potential metabolites were prepared by synthesis as shown in Schemes 3 and 4.

The *erythro*-hydroxybutanoic acid **4** was prepared from rofecoxib as shown in Scheme 3. The diol was treated with a slight excess of acetyl chloride ($Et_3N$/DMAP) to produce a mixture of acetates, which were separated by silica gel chromatography and further purified by trituration in ether and hexanes. Absolute proof of structure for the monoacetate was obtained by regenerating **1** through Jones oxidation followed by cyclization (LiOH then HCl).

Upon double bond reduction of the monoacetate compound, the two protons adopt an *anti* orientation ($J^3_{2\text{-}3} = 12$ Hz). This indicates that the side chains containing carbons 1 and 4 also have an *anti* orientation, preventing closure of the lactone ring. *erythro*-Hydroxybutanoic acid (**4**) was isolated as the sodium salt.

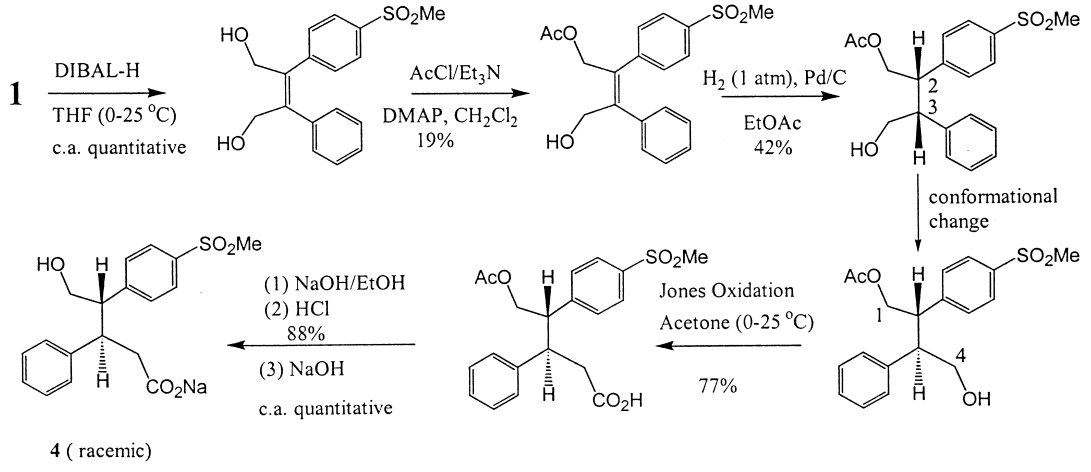



**Scheme 2.** Synthesis of the 5-hydroxy metabolite **2**. This reaction was originally developed by D. Tschaen, R. Tillyer, L. Frey, and U. Dolling.

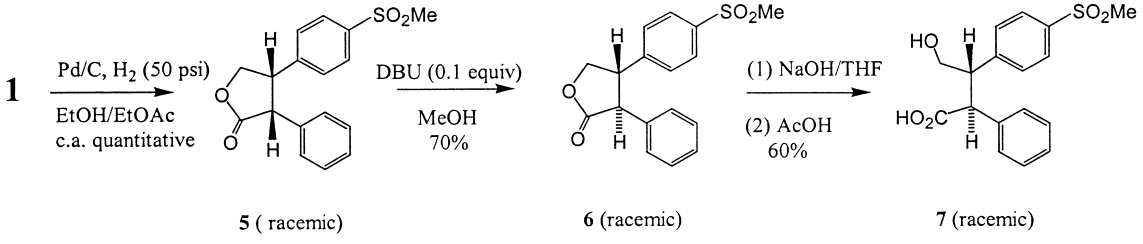
Racemic *cis*-lactone (**5**) was obtained from rofecoxib following catalytic hydrogenation (Scheme 4). This compound is quite unstable and readily isomerizes. Thus, treatment of this compound (**5**) with a catalytic amount of DBU in methanol produced the more stable *trans*-lactone **6**. Hydrolysis of the *trans*-lactone **6** gave the *threo*-hydroxybutanoic acid **7**.

The synthetic racemic *threo*-hydroxybutanoic acid **7** was shown to have the same chromatographic, $^1$H NMR and mass spectral characteristics as the isolated urinary dog metabolite. The four materials (**4**–**7**) obtained from chemical syntheses allowed confirmation of these metabolites in rat, dog, and human studies.[8,9] These compounds are difficult to quantify in in vivo samples due to interconversion between them, however, together they comprise the most significant pathway in humans.[8]

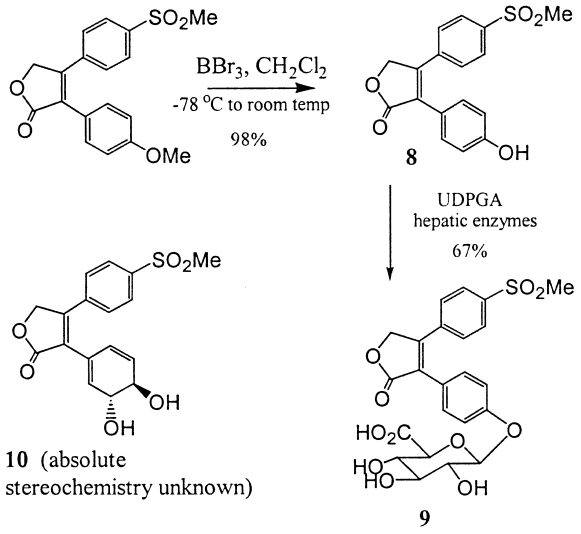
During development of rofecoxib, additional compounds derived from metabolism of the unsubstituted phenyl ring were observed (Scheme 5).[8,9] In humans these included an *O*-glucuronide of the 4′-hydroxy analogue (**9**) and the 3′,4′-dihydrodiol analogue (**10**). The 4′-hydroxy intermediate **8** was prepared by treatment of the 4′-methoxy compound[13] with $BBr_3$ as shown in Scheme 5. The 4′-*O*-glucuronide **9** was then prepared biosynthetically with rat microsomes and UDPGA by methodology analogous to that described above for racemic *O*-glucuronide **3**. Synthesis of the 3′,4′-dihydrodiol **10**, a very minor metabolite in humans,[8] was not attempted.




**Scheme 3.** Synthesis of the *erythro*-hydroxybutanoic acid metabolite of rofecoxib.




**Scheme 4.** Synthesis of *cis*- and *trans*-lactones and the *threo*-hydroxybutanoic acid metabolites of rofecoxib.




**Scheme 5.** Synthesis of the 4′-hydroxy metabolite **8** and the 4′-*O*-glucuronide **9**. Structure of metabolite **10**.

All of the biosynthetic and synthetic derivatives were tested in the human whole blood assays for COX-1 and COX-2 inhibition[3] and the results are reported in Table 1. The hydroxylated compounds **2** and **8** show some inhibitory effects on COX-2, however, they are an order of magnitude less potent than rofecoxib. The 4′-hydroxy derivative (**8**) showed some moderate inhibition of COX-1 activity. Although this material is likely a metabolite en route to the 4′-*O*-glucuronide, it was not detected in human biological fluids,[8] which suggests that it is a transient species that never builds up in concentration. Furthermore, the excreted 4′-*O*-glucuronide **9** accounts for $< 5\%$ of the dose.[8]

## Conclusion

Of the potential metabolites reported in Table 1, all but the 4′-hydroxy derivative (**8**) have been observed in humans.[8] The only human metabolite that shows any effect on COX-2 activity is the 5-hydroxy metabolite (**2**) with an $IC_{50}$ 17-fold less than the $IC_{50}$ of rofecoxib. None of the human metabolites showed measurable effects on COX-1 activity. These metabolites are, therefore, not anticipated to contribute to the therapeutic benefits of COX-2 inhibition, nor any of the potential effects associated with COX-1 inhibition.

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