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Facile air oxidation of the conjugate base of rofecoxib (Vioxx™), a possible contributor to chronic human toxicity

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Abstract—The COX-2 inhibitor rofecoxib (Vioxx™, **1**) ionizes under physiological conditions to an anion that reacts readily with dioxygen to form inter alia the reactive maleic anhydride derivative **6**, a potentially toxic compound.

The development of selective inhibitors of the inducible cyclooxygenase-2 (COX-2) has been one of the salient advances of recent years for the treatment of pain and inflammatory disease such as osteoarthritis.[1–4] Unlike older antiinflammatory agents (e.g., aspirin, indomethacin, ibuprofen, and naproxen), which inhibit both COX-2 and the constitutively expressed cyclooxygenase-1 (COX-1), the selective COX-2 inhibitors relieve pain with minimal gastric erosion that can result from the inhibition of cytoprotective COX-1-dependent synthesis of PGE_2 in the gastric mucosa.[2–4] However, the recent finding of significant elevation of adverse cardiovascular events in people already at risk has triggered the withdrawal from use of one of the most widely used COX-2 inhibitors, rofecoxib (Vioxx™).[5] The recall of Vioxx follows the publication of an extensive series of articles that argued in favor of an associated cardiovascular risk.[6–9] The issue was subsequently decided by the analysis of the data from the APPROVe trial that was intended to test for rofecoxib (25 mg daily) protection of recurrence of colorectal polyps. That study of 2000 patients demonstrated that the excess incidence of cardiovascular events was statistically significant for rofecoxib versus placebo (7.5 events per 1000 patients on placebo and 15 events per 1000 patients on rofecoxib). The increased risk only began to appear after 18 months of treatment and became clear after 24 months, causing early termination of the planned 36-month trial.

The withdrawal of Vioxx has quickly been followed by suggestions that all selective COX-2 inhibitors should be suspect because the cardiovascular side effects of Vioxx are common to the COX-2 class and mechanism related rather than compound specific.[5b,10,11] The argument for a COX-2 class-based adverse cardiovascular effect may be summarized as follows. Whereas the expression of COX-1 varies only modestly in a normal adult over a several day period (roughly up to 2-fold), the expression of COX-2 is much more highly variable (up to approximately 20- to 30-fold). COX-2 plays a significant role in regulating the tone of the vasculature since it produces the biochemical precursor (the endoperoxide PGH_2) for the vasodilating eicosanoid prostacyclin (PGI_2). Thus PGI_2 helps to maintain adequate blood flow and to compensate for arterial narrowing, platelet aggregation or other events, which tend to reduce circulation. The expression of COX-1 in platelets leads to increased formation of the platelet-aggregating eicosanoid thromboxane A_2 (TxA_2). Whereas COX-1 inhibitors act to decrease TxA_2 synthesis in platelets, COX-2 inhibitors do not, because platelets express COX-1 but not COX-2. The argument has recently been extended by some to the conclusion that all selective COX-2 inhibitors carry additional cardiovascular risk in patients with coronary artery disease because they are more subject to the catastrophic cascade of events that can result from impaired blood flow and hypoxia, especially of cardiac muscle.

A problem with this explanation is that the older nonsteroidal antiinflammatory agents such as ibuprofen and naproxen, which are still in use on a massive scale and which are non-specific inhibitors of both COX-1

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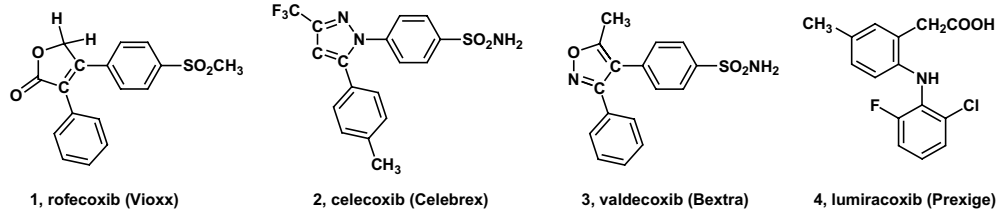


Figure 1.

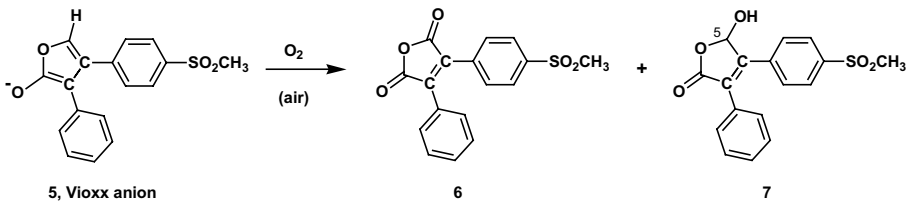
and COX-2, do not appear to cause a significant increase in cardiovascular risk. It is not easy to understand the relative cardiovascular safety of these drugs (excluding their well-known gastro-intestinal adverse effects) if there were really a COX-2 class effect, because at the doses often used with these agents there is a high level of COX-2 inhibition.

The purpose of this note is to describe a chemical property of rofecoxib that sets it apart from the other currently used COX-2 inhibitors. The chemical structures of rofecoxib (Vioxx, **1**), celecoxib (Celebrex™, 2), valdecoxib (Bextra™, **3**) and lumiracoxib (Prexige™, **4**) are shown in Figure 1.[12] Rofecoxib is a weak acid (pK_a ca. 12) and as a consequence it undergoes facile H/D exchange of the protons gamma to the butenolide carbonyl in D_2O or deuterated serum at 37 °C ($t_{1/2}$ approximately 7 min) via the corresponding conjugate base, **5**.[13] This anion is very reactive toward atmospheric O_2. When the anion is generated in THF (e.g., with 1 equiv of *n*-BuLi), it reacts rapidly with O_2 from air even at 0 °C giving a deeply colored solution within a few minutes that gradually becomes yellow-brown with the formation of a mixture of oxidation products. The principal product, which is readily isolated in pure form by silica gel chromatography, is the maleic anhydride **6**, mp 140–142 °C.[14] Also isolated in lesser amount is the γ-hydroxy butenolide **7**. When rofecoxib **1** is stirred in air in H_2O–THF solution at ambient temperature with 1 equiv of lithium hydroxide, it is also converted to **6** and **7**, with the former undergoing concomitant hydrolysis to the corresponding maleic acid. It has previously been reported that rofecoxib is also readily oxidized by O_2 at room temperature in ethyl acetate solution to give the γ-hydroxy butenolide **7** in the presence of powdered charcoal (92% yield).[15] Although **7** has been reported as an in vivo[16] and in vitro[17] metabolite of rofecoxib, to the best of our knowledge the maleic acid and maleic anhydride (**6**) products have not been.[18]

On the basis of the facile oxidation of the rofecoxib anion **5** and its presence in small amounts in equilibrium with **1** under physiological conditions, we think it likely that there is some degree of spontaneous oxidation of Vioxx as it circulates through the vasculature and oxygenated tissues in vivo. Therefore, it is also reasonable to expect that some of the maleic anhydride **6** would be formed in vivo. Although a portion of this oxidation product may simply react with water to form the corresponding maleic acid, some fraction may survive long enough to react with nucleophilic groups of biomolecules and tissue, especially amino groups. The consequences of this may be a low level chronic toxicity that is cumulative and possibly dangerous over periods of many months. It is perhaps not irrelevant that the cardiotoxicity of Vioxx was not apparent from short-term (one year or less) studies.

Studies of the fate of [14C] rofecoxib in healthy human subjects (administered orally, 125 mg) have revealed that 71.5% of the radioactivity ingested could be recovered from the urine and 14.2% was found in the feces.[16] The balance of the radioactivity (ca. 15%) was not accounted for. This result leaves open the possibility that some of the labeled agent might linger in the body for an extended period of time, which would be consistent with the possibility of conversion via **6** and covalent coupling to biomolecules and tissues as summarized above. A more detailed study of the fate of radio-labeled rofecoxib in vivo with experimental animals would provide a simple and critical test of this scenario.

It is important to note that the equilibration of rofecoxib with its anion and the high susceptibility of that anion (**5**) to air oxidation under physiological conditions is not a property shared by the two COX-2 inhibitors, which are currently in widest use, celecoxib and valdecoxib, which are not subject to air oxidation. This clear difference between **1** and **2** or **3** has been entirely overlooked in the medical and general literature, which tend to regard the three substances as equivalent. We believe that the basis of low level chronic toxicity of **1** that is disclosed herein is at least as plausible as the class effect argument that seems to be widely held.[5b,10,11] In view of the fact that many individuals who suffer from arthritis pain benefit from COX-2 inhibitors with lower risk of



gastric erosion, and the fact that COX-2 inhibitors may have other medical uses,[19] it seems inadvisable to assume that all members of this class are equivalent and too risky for human patients.[20]

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18. The experimental details of the air oxidation of rofecoxib are as follows. A solution of rofecoxib (**1**) (120 mg, 0.4 mmol) in THF (3 mL) was treated with 1 equiv of aqueous LiOH solution (16.4 mg, 0.4 mmol dissolved in 1 mL of H_2O) at 23 °C. After stirring the reaction mixture in air for 4 h at room temperature, THF was removed in vacuo and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine and dried over Na_2SO_4. The solvent was removed in vacuo and the residue was purified by column chromatography (silica gel, ethyl acetate–hexane, 1:1) to give the γ-hydroxy butenolide **7** (~5 mg). Then the aqueous layer was adjusted to pH 3 with HCl and the solution was concentrated under reduced pressure. The residue was extracted with ethyl acetate (4×) and the organic extract was washed with water, brine and dried over Na_2SO_4. The solvent was removed in vacuo and the residue was purified by column chromatography (silica gel, ethyl acetate–hexane, 1:1) to give the maleic anhydride **6** (30 mg). Data for compound **6**: $R_f = 0.90$ (75% ethyl acetate in hexane). Mp = 140–142 °C; FTIR (film) ν_{max}: 3095, 3070, 2929, 1831, 1764, 1312, 1270, 1150, 773 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): δ 8.02 (2H, d, $J = 8.4$ Hz), 7.78 (2H, d, $J = 8.4$ Hz), 7.55 (3H, m), 7.49 (2H, m), 3.13 (3H, s). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 164.43, 164.25, 142.69, 140.90, 136.04, 132.78, 132.26, 130.87, 130.02, 129.54, 128.23, 126.49, 44.59; HRMS (ESI) calcd for $C_{17}H_{13}O_5S$ 329.0484, found 329.0481 $(M+H)^+$. Data for compound **7**: (unstable, slowly decomposes) $R_f = 0.80$ (ethyl acetate). FTIR (film) ν_{max}: 3064, 3025, 2927, 1760, 1302, 1283, 1146, 959 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.88 (2H, d, $J = 8.0$ Hz), 7.64 (2H, d, $J = 8.0$ Hz), 7.40 (5H, m), 6.52 (1H, s), 3.08 (3H, s). HRMS (ESI) calcd for $C_{17}H_{14}O_5S$ 331.0640, found 331.0637 $(M+H)^+$.
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20. The material in this article has been disclosed to the US FDA.