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Tetrahedron Letters
Tetrahedron Letters 46 (2005) 3533–3536

A novel oxidative degradation pathway of indomethacin under the stressing by hydrogen peroxide

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Received 23 February 2005; revised 9 March 2005; accepted 11 March 2005
Available online 1 April 2005

Abstract—The structures of two oxidative degradation products of indomethacin were determined and their formation through a novel oxidative degradation mechanism is proposed. The synthesis of one of the degradation products is described.

Indomethacin is a nonsteroidal anti-inflammatory drug that is prescribed to mainly treat rheumatoid arthritis and pain. The major degradation pathway of the drug substance is the hydrolysis of the amide linkage leading to the formation of 4-chlorobenzoic acid and 2-methyl-5-methoxyindole-3-acetate.[1] However, no detailed oxidative degradation studies have been reported according to our survey. In order to look for potential oxidative degradation products, we performed oxidative stressing by using hydrogen peroxide as the oxidant. In this paper, we report a novel oxidative degradation pathway of indomethacin revealed from the study. In addition, a synthetically useful preparation procedure is described for one of the two major oxidative degradation products.

To carry out the oxidative degradation, indomethacin was dissolved in an aqueous mixture containing 0.23% of hydrogen peroxide and the resulting solution was heated at 80 °C for 75 min.[2] Two major degradation peaks were observed eluting prior to the indomethacin peak in the HPLC analysis of the reaction solution (Fig. 1).[3] LC–MS analysis[4] of the solution indicated that the first eluting degradation product (RRT 0.64)[5] had a molecular weight of 330, or $MW_{indo} + 16 - 44$ (where MW_{indo} is the molecular weight of indomethacin, 358) while the later eluting degradation product (RRT 0.73) had a molecular weight of 374 or $MW_{indo} + 16$. These values appear to imply that RRT 0.73 corresponds to a species that had an oxygen incorporated into indomethacin and a subsequent decarboxylation of this species might lead to RRT 0.64. Nevertheless, heat stressing the collected RRT 0.73 fraction did not yield any RRT 0.64, suggesting that the two species may share a common intermediate but one is not the precursor of the other. Based upon the known chemistry of the indole ring, the initially formed oxygenated species is most likely an indole-2,3-epoxide (**1**) as shown in Scheme 1. According to the work published by Zhang and Foote,[6] such an epoxide is not stable at room temperature and would be expected to rearrange into several secondary degradation products, among which a major one would be formed through a 2,3-alkyl shift concurrent with the cleavage of the epoxide ring (Scheme 1, route a). Following up on this lead and the molecular weight information obtained from the LC–MS study, the most probable structures (**2** and **3**, Scheme 1) were then proposed for the two degradation products (RRT 0.64 and RRT 0.73), respectively. In order to confirm the proposed structures (**2** and **3**), additional materials were needed for NMR structure determination. Hence, indomethacin was treated with 3-chloroperoxybenzoic acid in methylene chloride, a well known reagent for epoxide preparation, to see whether this procedure would generate the two degradation products in large enough quantities for the NMR study.[7] A large white precipitate

Keywords: Indomethacin; Indole; Oxidation; Degradation; Hydrogen peroxide; Epoxide; Decarboxylation.
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doi:10.1016/j.tetlet.2005.03.064

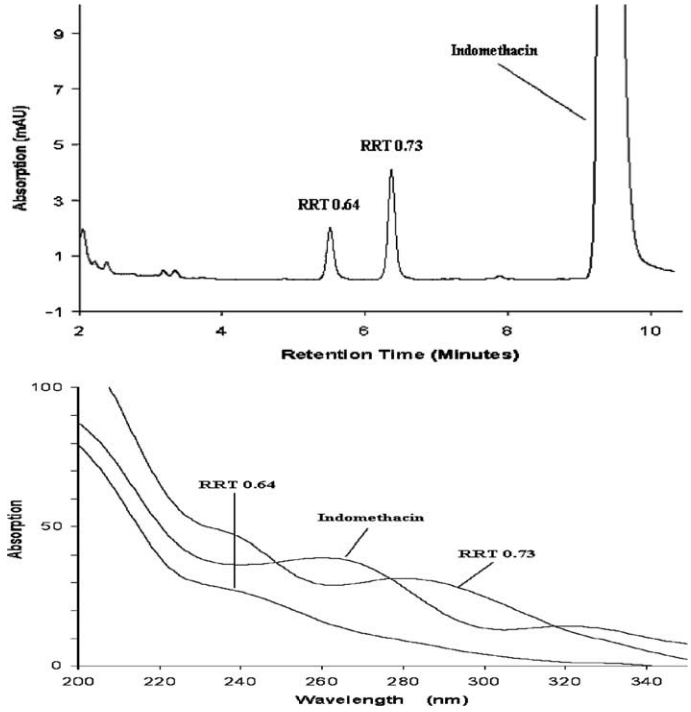
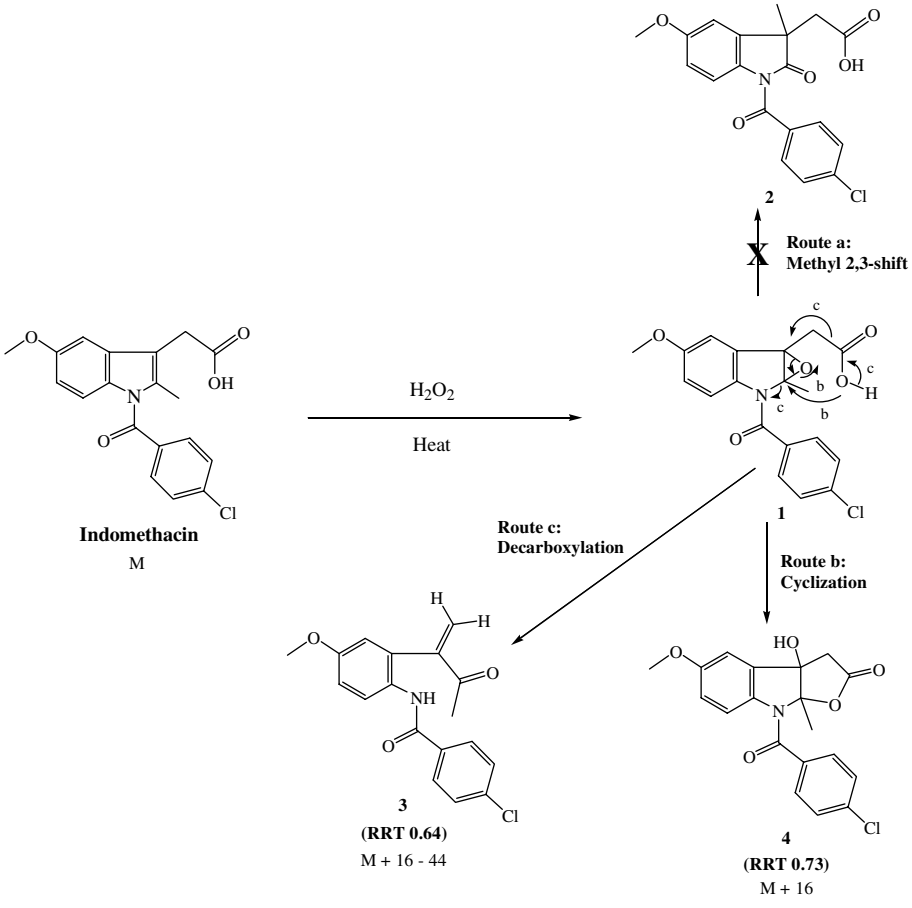


Figure 1. Upper: HPLC analysis of indomethacin solution in methanol/acetonitrile/water (1/2/3, v/v/v) stressed with 0.23% hydrogen peroxide under 80 °C for 75 min. Lower: UV scans of indomethacin and its two main degradation products at RRT 0.64 and 0.73.



Scheme 1. Oxidative degradation of indomethacin under stressing by hydrogen peroxide.

occurred during the course of the reaction and isolation of the material was achieved through filtration. The material was then dried and HPLC analysis indicated that it was a pure compound. Moreover, this material co-eluted with RRT 0.73, which was confirmed by analysis of the original aqueous oxidation solution[2] spiked with the material. Furthermore, a comparison of their UV scans with photo-diode array UV detector (Fig. 1) confirmed that they were the same species.

^{1}H NMR analysis of the material, nevertheless, showed that the proposed structure (**2**) was not completely compatible with the observed spectrum. Further analyses of the compound with 2D carbon–proton correlation techniques (HMQC and gHMBC) suggested that the correct structure was the one with a tricyclic framework (**4**, Fig. 2).[8] Apparently, the carboxyl attacked the 2-position which resulted in the opening of the epoxide ring and the concomitant formation of the lactone ring (Scheme 1, route b). A search for the tricyclic structure in the literature indicated that it was previously reported by Hirobe's group when they performed a drug metabolism studies on indomethacin using chemical P-450 model systems[9] as well as a rat liver microsomal system.[10]

While compound **4** (RRT 0.73) is quite stable at room temperature and can be readily made in large quantity,[7] compound **3** (RRT 0.64) is relatively unstable and various attempts to synthesize it in quantity were unsuccessful. It became necessary to isolate compound **3** from the original aqueous hydrogen peroxide stressing system by using preparative HPLC.[11] A small amount of compound **3** was then isolated and subjected to both 1D and 2D NMR analyses as well as high resolution mass determination;[12] all data gathered (Fig. 3) supported the proposed structure (**3**). The most obvious spectroscopic features are the two geminal protons occurring at 6.38 and 6.00 ppm as singlets with both attached to the same carbon at 128.3 ppm, thereby, indicating an *exo*-methylene unit as shown in Figure 3. ROESY cross-peaks were observed between 6.38 ppm singlet and the acyl methyl group at 2.38 ppm. In this case, decarboxylation was accompanied by a simultaneous opening of the epoxide ring, which in turn resulted in the breakage of the N–C bond between the 1 and 2 positions (Scheme 1, route c).

In summary, we have described a novel oxidative degradation pathway for indomethacin when it is stressed with hydrogen peroxide. One of the oxidative degradation products (**4**) was also observed previously as a metabolite when indomethacin was subjected to biotransformation with rat microsomal system and chemical P-450 model systems involving a porphyrin iron

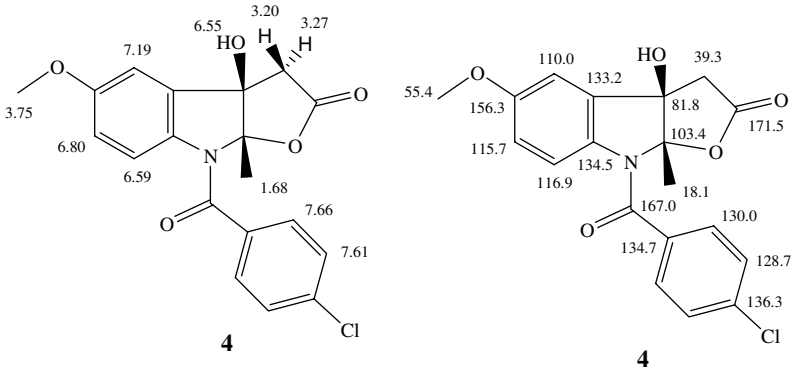


Figure 2. Proton (left) and carbon (right) chemical shift assignments of compound **4** based on 1D and 2D (HMQC and gHMBC) NMR experiments (DMSO-d_6; 25 °C). For simplicity, the other enantiomer is not shown.

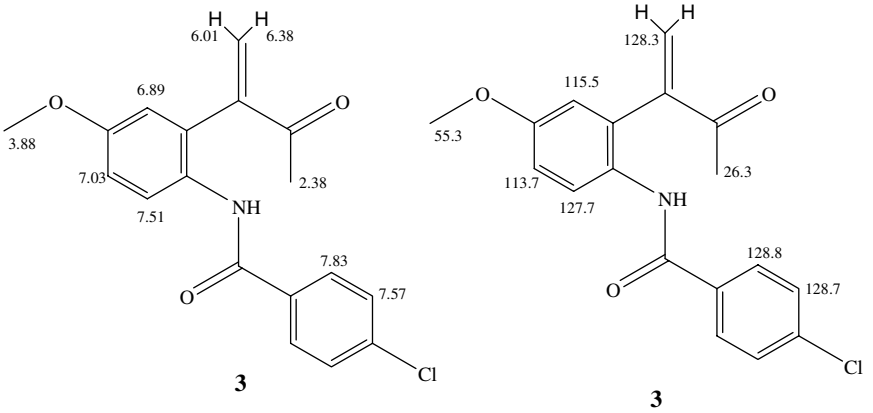


Figure 3. Proton (left) and carbon (right) chemical shift assignments of compound **3** based on 1D and 2D (HMQC) NMR experiments (CD_3CN; 25 °C). Only protonated carbons were assigned due to a limited sample amount.

moiety.[9,10] Another degradation product (**3**), a result of novel oxidative decarboxylation, has not been previously reported. In addition, we have developed a synthetic procedure that can produce compound **4** in large quantity which can be used as reference standard for future monitoring of the degradation product. A survey of the literature also indicates that compound **4** is structurally similar to a key intermediate for physostigmine,[13] an indole alkaloid clinically used as anticholinesterase, which appears to render **4** useful in preparing physostigmine analogs. Physostigmine and its analogs have been reported as potential therapeutic agents for Alzheimer's disease.[14]

Acknowledgements

We would like to thank Dr. Damon C. Barbacci for assistance with the mass determination experiment on Micromass LCT mass spectrometer.

References and notes

1. O'Brien, M.; McCauley, J.; Cohen, E. *Anal. Prof. Drugs Sub.* **1984**, *13*, 211–238.
2. A solution of 1.6 mg of indomethacin in 12 mL of a solvent mixture (methanol/acetonitrile/water, 1/2/3, v/v/v) was mixed with 1 mL of a 3% hydrogen peroxide solution. The resulting mixture was heated at 80 °C for 75 min. After cooling to room temperature, aliquots of the solution were injected into the HPLC system described in Ref. 3 for analysis.
3. The HPLC analyses were performed on a Waters Alliance system consisting of a Model 2695 Separations Module and a Model 2996 photo diode array detector. The system was equipped with an Alltech Platinum EPS C8, 100 × 4.6 mm, 3 μm column. Elution was effected with a linear gradient generated between solution A (acetonitrile/methanol, 1/6, v/v) and solution B (0.1% formic acid in water): the percentage of solution A was increased from an initial 30% to 70% over a period of 15 min. The flow rate was 1.0 mL/min and the column was heated at 35 °C.
4. The LC–MS analyses were carried out on a TSP HPLC system interfaced to a Finnegan LCQ mass spectrometer. Experiments were performed in electrospray ionization (ESI)-positive ion mode. A flow rate of 1.0 mL/min directly in source (no post-column split) was used. The temperature of the capillary was set to 200 °C. The LC gradient is the same as outlined in Ref. 3.
5. RRT is 'relative retention time' of the peak relative to indomethacin which is defined as (retention time of the peak)/(retention time of indomethacin).
6. Zhang, X.; Foote, C. S. *J. Am. Chem. Soc.* **1993**, *115*, 8867–8868.
7. To a solution of indomethacin (503 mg, 1.41 mmol) in 15 mL of methylene chloride cooled in an ice-water bath was added dropwise a solution of 3-chloroperbenzoic acid (77% purity, 625 mg, 2.81 mmol) in 10 mL of methylene chloride over a period of 30 min. The resulting solution was allowed to warm to room temperature and stirred overnight. The white precipitate formed was collected by filtration. After drying in vacuo, a fine white powder was obtained (391 mg, 1.05 mmol, 74% yield): ^{1}H NMR (DMSO-d_6) δ 7.66 (d, 2H, J = 8.5 Hz), 7.61 (d, 2H, J = 8.5 Hz), 7.19 (d, 1H, J = 2.75 Hz), 6.80 (dd, 1H, J_1 = 2.75 Hz, J_2 = 9.16 Hz), 6.59 (br s, 1H), 6.55 (s, 1H), 3.75 (s, 3H), 3.27 (d, 1H, J = 17.5 Hz), 3.20 (d, 1H, J = 17.5 Hz), 1.68 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 171.5, 167.0, 156.3, 136.3, 134.7, 134.5, 133.2, 130.0, 128.7, 116.9, 115.7, 110.0, 103.4, 81.8, 55.4, 39.3, 18.1. IR (KBr) 3354, 1793, 1621, 1390, 1083 cm^{-1}.
8. NMR spectra were recorded on a Varian Unity Inova 600 NMR spectrometer equipped with a 5 mm triple resonance indirect detection probe (HCN). Standard VNMR (version 6.1C) pulse sequences were used for all experiments. The RRT 0.73 material was dissolved in DMSO-d_6 to which TMS was added as an internal chemical shift reference. Proton and carbon chemical shift assignments (Fig. 2) were made based on the results of proton 1D, HMQC, and gHMBC experiments performed at a sample temperature of 25 °C. The hydroxyl proton (6.55 ppm) exhibited a two-bond coupling to the 3-position carbon at 81.8 ppm and three-bond couplings to carbons at 133.2, 103.4, and 39.3 ppm. The methyl group protons were found coupled to the 2-position carbon at 103.4 (two-bond) and the 3-position carbon at 81.8 ppm (three-bond). The methylene protons (3.27 and 3.20 ppm) were non-equivalent due to the presence of stereocenters in the molecule. The methylene protons were found coupled to carbons at 171.5, 133.2, 103.4, and 81.8 ppm. The coupling from the 3.27 ppm proton to the 103.5 ppm carbon was stronger than the coupling to the coupling from the 3.20 ppm proton to the same carbon due to a more favorable dihedral angle. Irradiation of the 7.19 ppm proton in a difference NOE experiment produced a stronger enhancement in the 3.27 ppm proton than in the 3.20 ppm proton. Irradiation of the 1.68 ppm methyl group produced a strong enhancement in the hydroxyl proton (verified cis ring juncture) and an enhancement in the 3.20 ppm proton. The NOE results together with the heteronuclear couplings allowed their stereospecific assignment as shown in Figure 2.
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10. Hirobe, M. *Pure Appl. Chem.* **1994**, *66*, 729–736.
11. The stressing condition is similar to what is described in Ref. 2 except that a more concentrated indomethacin solution was used (0.5 mg/mL in methanol/acetonitrile/water, 1/2/3, v/v/v) and final hydrogen peroxide concentration was 0.3%. Aliquots of the stressed solution were injected into a preparative Waters HPLC system consisting of a Model 600 pump and controller unit and a model 2996 photodiode array detector. The system was equipped with a Waters Xterra RP18, 19 mm × 100 mm, 5 μm column. Elution was effected with a linear gradient generated between solution A (acetonitrile/methanol, 1/6, v/v) and solution B (0.1% formic acid in water). The percentage of solution A was increased from an initial 40% to 90% over 15 min. The flow rate was 25 mL/min. and column was maintained at ambient temperature. The fractions corresponding to RRT 0.64 (compound **3**) were collected and the pooled fractions were then dried by evaporation in vacuo.
12. Compound **3** HPLC isolate was dissolved in 160 μL of CD_3CN and transferred to a 3 mm NMR tube. NMR experiments were carried out at 25 °C on a Varian 500 MHz spectrometer equipped with a 5 mm HCN Cold Probe. 1D ^{1}H, 2D TOCSY, ROESY, and HMQC data sets were acquired and analyzed to elucidate the structure. High resolution mass on Micromass LCT time-of-flight mass spectrometer: Calcd for [$C_{18}H_{16}NO_3Cl$+H^+], 330.0897. Found: 330.0978.
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