Synthesis of an Albendazole Metabolite: Characterization and HPLC Determination

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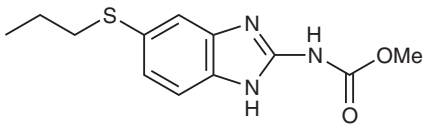
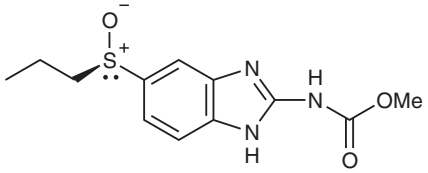
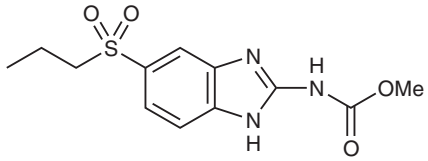
Sulfoxides are remarkable molecules with interesting biological and chemical properties that can be readily obtained by oxidation of the corresponding sulfides *(1).* Sulfoxides are present in many drugs such as omeprazole, the proton pump inhibitor *(2),* sulindac, the non-steroidal anti-inflammatory drug *(3),* and modafinil, a drug used for the treatment of narcolepsy *(4, 5a).* Sulfoxides are particularly interesting because they can be chiral *(5).*



albendazole, **1**

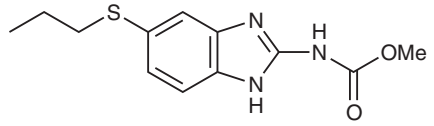


(±)-albendazole sulfoxide, **2**

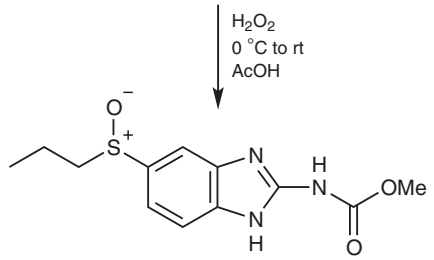


albendazole sulfone, **3**

Figure 1. Albendazole and its metabolites.



albendazole, **1**



(±)-albendazole sulfoxide, **2**

Scheme I. Synthesis of albendazole sulfoxide.

Albendazole, **1,** belongs to the family of benzimidazole drugs and is used as an anthelmintic in both human and veterinary medicine. It has a broad spectrum of activity against nematode parasites of the intestinal tract such as ascariasis, intestinal capillariasis, enterobiasis, trichuriasis, and hookworm infections. Albendazole is first metabolized to sulfoxide **2** by hepatic microsomal cytochrome P-450 in the liver where both (*S*) and (*R*) isomers are formed. The sulfoxide **2** is further oxidized to the sulfone **3** (*6*; Figure 1). Albendazole sulfoxide, **2,** and albendazole, **1,** are both as biologically active while the sulfone metabolite, **3,** is inactive *(7).*

We report an integrated laboratory project for undergraduate students in medicinal chemistry laboratory that focuses on organic synthesis, nuclear magnetic resonance (NMR) spectroscopy, and high-performance liquid chromatography (HPLC) analysis. The students synthesize albendazole metabolite **2** by oxidation of compound **1** using H_2O_2 in acetic acid *(7, 8)* (Scheme I). They use HPLC to analyze the crude reaction and determine the concentration of sulfoxide **2.** Students acquire experience in laboratory manipulation and in the use of common techniques such as thin-layer chromatography (TLC). They analyze ^{1}H NMR, infra red, and mass spectroscopy spectra provided by the instructor

Experimental Overview

Students are divided into groups to perform the experiments. There are two students per group and three groups work simultaneously on the same experiment. The rest of the class works on other experiments. By the end of the semester all students had completed all of the laboratory experiments, a total of three. The experiment can be completed in a total of three lab periods (4 to 5 hours each). In the first lab period, the albendazole oxidation reaction is performed and monitored by TLC, usually requiring 4 h. The second lab period is used to precipitate the sulfoxide and discuss the spectroscopic data of the compound. The third lab period is used to run HPLC determinations: calibration curve and crude reaction purity. A "class discussion" is held to go through aspects about the oxidation and HPLC determination before students submit a brief report with yield, purity determinations, and the conclusions.

Theory and applications of HPLC should be an integral part of college chemistry curricula since many students will be using this technique in their professional work, especially in industry. Because of the short reaction time and simple workup, students have the opportunity to complete the experiment in three laboratory sessions.

Oxidation Reaction

A racemic mixture of albendazole sulfoxide, **2,** is formed by chemical oxidation of albendazole, **1,** at the thioether bridge (Scheme I). The reaction is performed adding 30% H_2O_2 (0.47 mL) to a solution of albendazole (1 g) in glacial acetic acid (8

mL) at 0 °C The mixture is allowed to reach room temperature. The oxidation reagent is the peracetic acid formed in situ *(9)*. After the reaction is completed, the pH is raised to 6, using 10 M NaOH solution. This pH is the optimum for sulfoxide **2** precipitation. The precipitate is vacuum filtered, dried, and used for HPLC determination. Routine TLC is used to check product formation and to evaluate the need for additional H_2O_2. The reaction is completed in 3 to 4 hours. Students have the opportunity to discuss spectroscopic methods for characterization. Reported reaction yields are between 70–90% and the purities range between 70–99%.

The results obtained in the oxidation reaction described above are useful to discuss the following:

- Why sulfoxides are chiral and nitrogen is not. Students realize that carbon is not the only possible chiral center. Nitrogen can be chiral, in theory, if its pyramidal inversion is limited or locked. Students can propose methods to obtain a single enantiomer using kinetic resolution, diasteromeric resolution, biocatalysts, or asymmetric oxidation such as the Sharpless methodology *(10)*. Analytical and semipreparative resolution of albendazole sulfoxide enantiomers can be performed using chiral HPLC according the literature *(11)*.
- Whether the pharmacological activity of albendazole sulfoxide is influenced by the sulfur configuration. If so, why? Apparently both isomers are formed in equal amounts in the body, but (*R*)-(+) isomer metabolizes slower than (*S*)-(−) isomer, reaching higher concentration in plasma *(6)*.
- The dependence of the albendazole sulfone, **3,** formation on the reaction conditions (temperature, equivalents, concentration). This can be explored by different groups of students running the reaction at 40 °C and adding 2 equivalents of H_2O_2. The groups can compare their results to illustrate these differences.
- The advantages (no waste, cheaper, easy-to-handle) of the use of H_2O_2 instead other oxidants such as *m*-chloroperbenzoic acid *(7),* $NaIO_4$, $NaClO_2$, sodium perborate ($NaBO_2 \cdot H_2O_2 \cdot 3H_2O$), and $KMnO_4$ *(9)*.
- The pH–solubility relationship for albendazole sulfoxide, **2.** The U-shaped pH–solubility profile in aqueous solutions indicates that it is an ampholyte with pK_a values of 3.4 for the basic group and 9.8 for the non-basic nitrogen *(12)*.
- The diastereotopic effect, the influence of the chiral sulfoxide on the protons adjacent to the sulfur, in the albendazole ^{1}H NMR spectrum.

HPLC Determination

Liquid chromatography is performed using a modified methodology reported by Gomes and Nagaraju *(13)*. A reverse-phase phenyl column (we used a ZORBAX SB-Phenyl 4, 6 mm × 25 cm column) is used as stationary phase and the mobile phase is a mixture of 50:50 v/v of methanol and trichloracetic acid in H_2O (6 mM, pH 2.2). The column was eluted using an isocratic mobile phase with at a flow rate of 1.0 mL min^{-1}, and products were identified by UV detection at 290 nm. Solutions of reference compounds **1, 2,** and **3** (using the eluent as solvent) are used to determine retention times for each compound. A typical chromatogram of the reaction mixture, in which the three compounds are present in varying amounts, is shown in Figure 2. Under these conditions, the retention times are 8.0 ± 0.5 min for albendazole sulfoxide, **2**, 9.1 ± 0.2 min for albendazole sulfone, **3,** and 19.8 ± 0.2 min for albendazole, **1**. Standard solutions of albendazole sulfoxide are used to construct the calibration curve spanning the concentration range 3×10^{-6} M to 2.0×10^{-5} M. The run time on the HPLC instrument (given optimum conditions) is 3 hours (includes equilibration times and five injections at 20 minutes each).

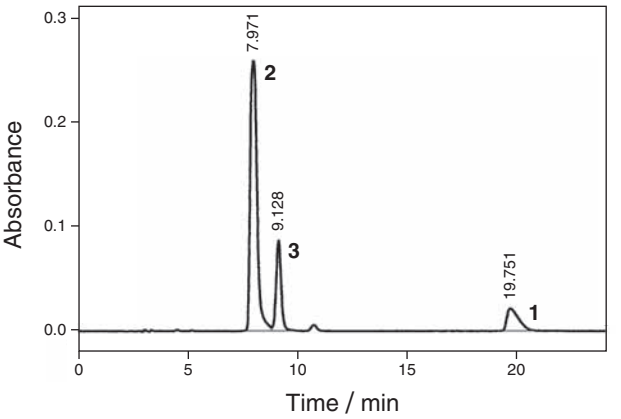


Figure 2. Chromatogram of compounds **1, 2, 3** mixture.

Hazards

Hydrogen peroxide is a strong oxidizer, corrosive, and harmful if inhaled. It causes eye and skin burns and severe digestive tract irritation. Vapor can detonate above 70 °C. Precaution should be taken when preparing albendazole sulfone; the temperature should never exceeds 40° C. Acetic acid is strongly corrosive and causes serious burns. Hexane is highly flammable, harmful by inhalation, an irritant, and may cause central nervous system depression. Chloroform is a suspect carcinogen and mutagen. Sodium hydroxide is very corrosive, causes severe burns, and is harmful by skin contact or by inhalation of dust. Methanol is a flammable liquid and may cause central nervous system depression. Trichloroacetic acid causes eye and skin burns and digestive and respiratory tract burns.

Conclusion

We report a biomimetic synthesis using an environmentally friendly methodology to obtain albendazole sulfoxide metabolite. HPLC analysis, which was integrated into our undergraduate medicinal chemistry course, is used to determine the metabolite's purity. This experiment can be carried out by students who have previously been trained in organic chemistry laboratory manipulations and have analytical knowledge. The experiment is well-liked among the students. They appreciate the practical training in HPLC, which is an important skill in today's chemical industry, and learning medicinal chemistry and synthesis.

The reaction and HPLC determination of albendazole sulfoxide illustrate important concepts: (i) green oxidation, (ii) thioether metabolism and chiral sulfoxides, (iii) controlled oxidation conditions to avoid sulfone formation, (iv) TLC

monitoring and comparative polarities of thioethers, sulfoxides, and sulfones, (v) HPLC methodology, analyses of the reaction mixture, construction of a calibration curve to determine sulfoxide purity, and (vi) diastereotopic influence of the sulfoxide on the protons adjacent to the sulfur through ^{1}H NMR analysis. Extra time can be used to discuss the spectral data of the product and the stereochemistry concepts involved in the procedure. Proper care of our environment dictates that we should minimize waste in our teaching labs; therefore, we recommend that chromatographic eluents could be used to prepare crude reaction solutions.

Acknowledgments

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