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Research Article

An improved synthesis of isotope labeled 6-hydroxychlorzoxazone

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Summary

[$^{13}C_6$]6-Hydroxychlorzoxazone, a major P450 metabolite of chlorzoxazone, was synthesized from [$^{13}C_6$]benzene in an overall 18% yield. An improved procedure for converting 4-chloro-6-nitrosoresorcinol to 6-hydroxychlorzoxazone was developed, with a yield more than double that previously reported in the literature. Copyright © 2006 John Wiley & Sons, Ltd.

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Key Words: 6-hydroxychlorzoxazone; 4-chloro-6-nitrosoresorcinol; chlorzoxazone; stable isotope

Introduction

P450 enzymes are a group of mixed function monooxidases involved in the oxidative metabolism of many different compounds, including drugs, environmental pollutants, steroids, prostaglandins, and fatty acids. Chlorzoxazone, a compound used therapeutically as a central acting muscle relaxant, was found to be oxidized to 6-hydroxychlorzoxazone in human liver microsomes.[1] Our own research at Novartis in this field created the need for stable isotope labeled 6-hydroxychlorzoxazone with a molecular weight at least 4 mass unit higher than the non-labeled compound.

A quick literature search revealed that synthesis of both of the non-labeled and the stable isotope labeled 6-hydroxychlorzoxazone have been reported. The synthesis of C-13 labeled 6-hydroxychlorzoxazone was accomplished through a 5-step preparation starting from C-13 labeled phenol. Unfortunately, the reported yield was only less than 2%, a result caused by the very

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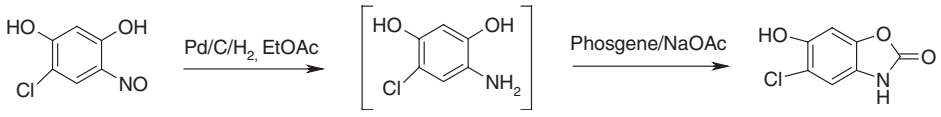

Figure 1. Synthesis of 6-hydroxychlorzoxazone

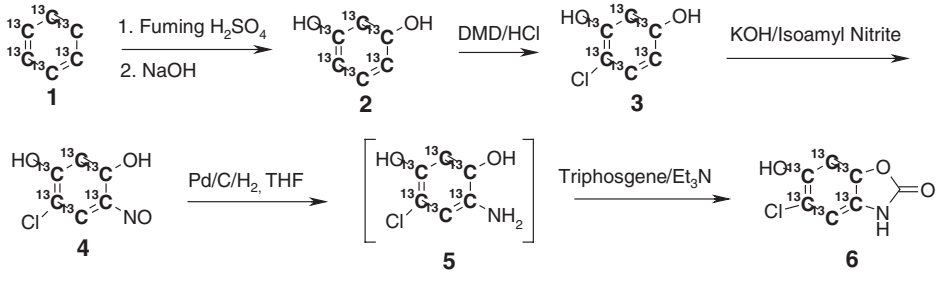

Figure 2. Synthesis of [^{13}C]-labeled 6-hydroxychlorzoxazone

low yield (7%) in the key step, the enzymatic hydroxylation of chorzoxazone.[2] Synthesis of non-labeled 6-hydroxychlorzoxazone was achieved by converting 4-chloro-6-nitrosoresorcinol to 6-hydroxychlorzoxazone (Figure 1).[1,3] The reported yields for this synthesis were quite different: while one author claimed 32%,[3] the other one only reported 16%,[1] both on multi-gram scales. Since we have developed a way to produce [$^{13}C_6$]resorcinol in our lab, we decided to carry out the synthesis by a similar route to the non-labeled synthesis.

Results and discussion

The synthesis of [$^{13}C_6$]6-hydroxychlorzoxazone in our lab is outlined in Figure 2.

[$^{13}C_6$]Benzene (**1**) was converted to [$^{13}C_6$]resorcinol (**2**) in 51.7% yield, first by treating with fuming sulfuric acid, and then by fusing with sodium hydroxide at high temperature. Chlorination of phenolic compounds is usually troublesome and mixtures of multi-chlorinated products are often formed following treatment with chlorine. A recent report claimed selective mono-chlorination of phenolic compounds when treating a solution of a phenol with excess of DMD (dimethyldioxirane), in the presence of 1 equivalent of hydrogen chloride.[4] One of the advantages of this method is that the hydrogen chloride can be generated italics from diluted sulfuric acid and sodium chloride, which can be accurately weighed so that exactly 1 equivalent of active chlorine can be generated. Synthesis of [$^{13}C_6$]4-chlororesorcinol (**3**) following this procedure was quite successful, we were able to achieve 76% yield of the

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desired mono-chlorination product [$^{13}C_6$]4-chlororesorcinol (**3**). A minor amount of [$^{13}C_6$]dichlororesorcinol was also detected (8.6%), even when the reaction was carried out strictly with only 1 equivalent of sodium chloride.

Our first attempt to prepare 4-chloro-6-nitrosoresorcinol following a literature procedure, by treating a solution of 4-chlororesorcinol and sodium ethoxide in ethanol with *n*-butyl nitrite,[3] was unsuccessful. We were not able to produce any detectable amount of 4-chloro-6-nitrosoresorcinol this way. Fortunately when the reaction was done with potassium hydroxide and isoamyl nitrite,[5] we were able to prepare [$^{13}C_6$]4-chloro-6-nitrosoresorcinol (**4**) in a reasonable yield (60%).

In the reported non-labeled synthesis of 6-hydroxychlorzoxazone,[1,3] 4-chloro-6-nitrosoresorcinol was first hydrogenated to produce 4-amino-6-chlororesorcinol, and then the solution of the 4-amino-6-chlororesorcinol was filtered to remove the palladium catalyst before reacting with phosgene. Our preliminary experiment with the reduction of 4-chloro-6-nitrosoresorcinol revealed that the reaction solution was almost colorless when hydrogenation was completed, but it turned to dark brown quickly when we tried to filter the reaction mixture, an indication that oxidative by-products had formed during filtration, even when it was done under a nitrogen atmosphere. We believe that this is one of the major factors which had led to low and un-reproducible yield. The other possible factor was the use of phosgene, a very reactive reagent. We believe that phosgene reacts non-selectively with both amino and hydroxyl groups in the molecule, although the nucleophilicity of the amino group in 4-amino-6-chlororesorcinol is stronger than the two hydroxyl groups. Improvements in yield can be achieved if we can eliminate the filtration step and if we can use a less active phosgene equivalent. It turned out to be the case: [$^{13}C_6$]6-hydroxychlorzoxazone (**6**) was produced in much higher yield when we eliminated the filtration step and replaced phosgene with triphosgen, a safer and milder phosgene equivalent.[6] Thus, [$^{13}C_6$]4-chloro-6-nitrosoresorcinol (**4**) was first catalytically hydrogenated in THF to the corresponding amine, and then without filtration to remove the catalyst, triethylamine and a THF solution of stoichiometric amount of triphosgene was added quickly with exclusion of oxygen. The reaction proceeded quickly, and after shaking for 30 min at room temperature, [$^{13}C_6$]6-hydroxychlorzoxazone (**6**) formed in a much better yield (75%). Initially we were also concerned about possible over reduction, resulting in the removal of chlorine from the benzene ring, but it turned out to be unnecessary because hydrogenation stopped under our reaction conditions (10% Pd/C as catalyst, THF as solvent, and under 50 psi hydrogen pressure) when 2 equivalents of hydrogen were consumed. No products from chlorine removal were detected.

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Experimental

Materials and methods

^{1}H NMR Spectra were recorded on a 500 MHz Bruker NMR spectrometer. Chemical shifts are reported in ppm relative to TMS. The LC-MS analysis was performed on a LCQ-Advantage mass spectrometer coupled with a Waters Alliance HPLC instrument. HPLC conditions: column, Phenomenex Polar RP-C18, 4μ, 4.6 × 150 mm; mobile phase A, 10 mM ammonium acetate with 0.5% acetic acid; mobile phase B, acetonitrile; gradient, 18 min from 80% A–20% B to 20% A–80% B; flow-rate, 1 ml/min; UV, 297 nm. The chemicals and solvents were reagent grade obtained from Sigma Aldrich without further purification. [$^{13}C_6$]Benzene was a Cambridge Isotope Labs product.

*[$^{13}C_6$]Resorcinol (**2**).* Fuming sulfuric acid (20%, 32 g) was added drop wise at room temperature to [$^{13}C_6$]benzene (10 g, 119 mmol) with stirring. The reaction temperature was raised to 60°C after the addition was complete. More fuming sulfuric acid (66%, 26 g) was added drop wise over 2 h and the resulting reaction mixture was stirred for 1 h at 90°C. The reaction mixture was then cooled down to room temperature, poured into water (250 ml), boiled, and neutralized with calcium carbonate (51 g). The hot solution was filtered and the filtrate was treated with sodium carbonate (13 g). Precipitate formed while adding sodium carbonate and was removed by filtration. The filtrate was concentrated to dryness to afford a solid (38.5 g) after drying under vacuum. The solid was then added portionwise to a melt of sodium hydroxide (41.5 g) and water (26 ml) at 200°C. The mixture was distilled under reduced pressure to afford a solid, which was heated at 320°C for 5 h, and then cooled to room temperature. Water (80 ml) was added to the reaction mixture, the suspension was acidified with conc. HCl, filtered through a thin layer of celite, and the filtrate was extracted with diethyl ether (6 × 30 ml). The combined diethyl ether extracts were dried over sodium sulfate, filtered, and evaporated to dryness to afford a dark tar material, which was distilled under high vacuum, heating with a heat-gun and collecting in an end-trap cooled at liquid nitrogen temperature to afford (**2**) as a yellowish solid (7.14 g, 52%). ^{1}H NMR ($CDCl_3$) δ 7.02 (m, d, J = 167 Hz, 1 H), 6.34 (m, d, J = 160 Hz, 2 H), 6.31 (m, d, J = 159 Hz, 1 H); MS (ESI-): m/z 115 ([M-H]$^-$).

*[$^{13}C_6$]4-Chlororesorcinol (**3**).* Preparation of dimethyldioxirane (DMD): Oxone (240 g, 390 mmol) was added from a solid addition funnel to a mixture of water (500 ml), acetone (360 ml), and sodium bicarbonate (120 g, 1428 mmol) cooled in an ice bath in 25 min. DMD, along with acetone, was distilled under slight vacuum (> 500 mmHg) to afford a slightly yellow solution (180 ml).

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Preparation of [$^{13}C_6$]4-chlororesorcinol (**3**) : [$^{13}C_6$]resorcinol (**2**, 600 mg, 5.2 mmol) was added to acetone (6 ml), followed by 10% sulfuric acid (5.2 ml) and sodium chloride (303 mg, 5.2 mmol). The resulting reaction mixture was stirred until a homogeneous solution was achieved and then the DMD solution (180 ml) was added all at once. The reaction mixture was stirred at room temperature for 10 min, concentrated to remove acetone, and extracted with diethyl ether (3 × 15 ml). The combined diethyl ether extracts were dried over magnesium sulfate, filtered, and evaporated. The brownish residue was purified by flash chromatography (silica gel column, eluted with hexane/diethyl ether 3/1) to afford pure [$^{13}C_6$]4-chlororesorcinol (**3**, 470 mg) as a colorless solid. An impure solid (205 mg) was also recovered. LC-MS analysis indicated that the impure material contained [$^{13}C_6$]4-chlororesorcinol (**3**, 60%) and [$^{13}C_6$]4,6-dichlororesorcinol (40%). This brings the total yield for [$^{13}C_6$] 4-chlororesorcinol (**3**) to 76%. ^{1}H NMR ($CDCl_3$) δ 7.16 (m, d, J = 173 Hz, 1 H), 6.54 (m, d, J = 160 Hz, 1 H), 6.39 (m, d, J = 160 Hz, 1 H); MS (ESI-): m/z 149 (100, [M-H]$^-$), 151 (33).

*[$^{13}C_6$]4-Chloro-6-nitrosoresorcinol (**4**).* To a solution of [$^{13}C_6$]4-chlororesorcinol (**3**, 460 mg, 3.07 mmol)in ethanol (2.4 ml) cooled in an ice bath was added a solution of potassium hydroxide (240 mg, 6 mmol) in water (0.47 ml), followed by isoamyl nitrite (422 mg, 3.6 mmol) drop wise with stirring. The reaction mixture was stirred at room temperature for 1 h before the pH of the solution was adjusted to 2 with 1 N hydrochloric acid. Precipitate formed during acidification and was collected by filtration. The filter cake was washed with 1/1 ethanol/water (3 × 1 ml) and water (3 × 1 ml). Crude [$^{13}C_6$]4-chloro-6-nitrosoresorcinol (**4**) was obtained (530 mg) after brief drying under vacuum. The crude (**4**) was passed through a short silica gel cartridge (Anologix, 4 g silica gel), eluting with hexane/ethyl acetate (6/4) to afford (**4**) as a yellow solid (330 mg, 60%). ^{1}H NMR (CD_3OD) δ 7.81 (d, J = 173, 1 H), 5.88 (d, J = 166, 1 H). MS (ESI-): m/z 178 (100, [M-H]$^-$), 180 (34).

*[$^{13}C_6$]6-Hydroxychlorzoxazone (**6**).* [$^{13}C_6$]4-Chloro-6-nitrosoresorcinol (**4**, 180 mg, 1 mmol) was dissolved in anhydrous THF (15 ml) in a hydrogenation bottle. Catalyst 10% Pd/C (50 mg) was then added. The reaction mixture was evacuated and purged with nitrogen. This process was repeated three more times in order to remove all air. The hydrogenation was carried out at 50 psi under hydrogen until calculated amount of hydrogen has been taken up (45 ml). Triethylamine (290 μl) was added, followed by a solution of triphosgene (104 mg, 0.35 mmol) in THF (1.5 ml) under nitrogen atmosphere. The resulting mixture was allowed to react for 30 min at room temperature, and then filtered through a thin layer of celite to remove catalyst. The filtrate was concentrated to afford a light brownish residue which was purified by flash

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chromatography (Analogix silica gel cartridge with 4 g silica gel, eluted with hexane/ethyl acetate 7/3) to afford pure [$^{13}C_6$]6-hydroxychlorzoxazone (**6**, 105 mg). A second batch (49 mg) of impure (**6**) was also recovered with 79% being the desired product when checked by HPLC. The total yield of (**6**) from (**4**) is thus 75%. ^{1}H NMR (CD_3OD) δ 6.99 (d, $J = 99$, 1 H), 6.67 (d, $J = 96$, 1 H). MS (ESI-): m/z 190 (100, [M-H]$^-$), 192 (33), and no non-labeled (M + 0) species was detected.

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