THE PREPARATION OF ORMETOPRIM LABELED WITH CARBON-14

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SUMMARY

The sulfonamide potentiator ormetoprim (6) has been prepared with a carbon-14 label for use in biological studies. The key intermediate, 2-methylveratraldehyde (4), was prepared by $^{14}CO_2$ carbonation of the Grignard reagent from 2-bromo-4,5-dimethoxytoluene (1) followed by esterification, reduction and manganese dioxide oxidation.

Key Words: 2-methylveratraldehyde-7-^{14}C, ormetoprim-benzyl-^{14}C.

Ormetoprim (6), a sulfonamide potentiator that is being increasingly used in animal science, has been prepared with a carbon-14 label suitable for use in pharmacokinetic and metabolic studies in a variety of species. The pyrimidine ring of ormetoprim could be labeled with carbon-14 at a late stage of the synthesis but the possibility of metabolic breakdown and subsequent loss of the label prompted us to choose the benzylic carbon atom as the carbon-14 carrier.

A Grignard reagent was prepared from 2-bromo-4,5-dimethoxytoluene (1) which was carbonated with carbon-14 dioxide to provide the labeled benzoic acid (2) in 84% yield. Several approaches from 2 to the aldehyde 4 were investigated and the cleanest product obtained in the highest yield resulted

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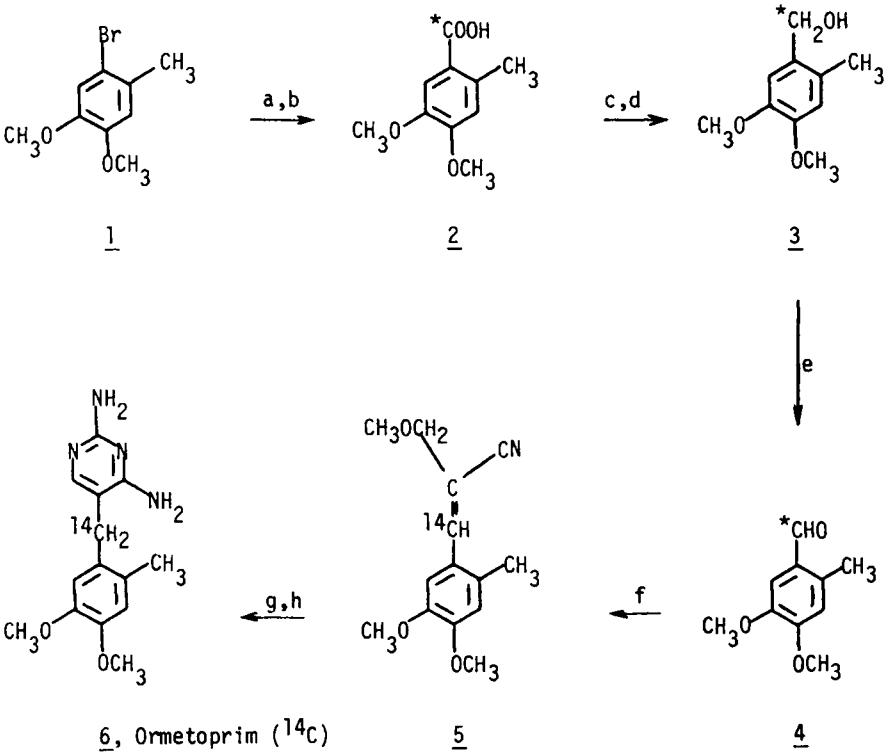

a, Mg; b, $^{14}CO_2$; c, CH_2N_2; d, $LiAlH_4$; e, MnO_2; f, $NaOCH_3$, $CH_3OCH_2CH_2CN$; g, $NaOCH_3$; h, $(H_2N)_2CNH$.

when the acid was first esterified, then reduced to alcohol 3 which in turn was oxidized to 4 with manganese dioxide. Overall yield for these two steps was 91%. Condensation of 4 with 3-methoxypropionitrile to yield 5 proceeded along the lines reported[1] for the nonradiochemical synthesis of ormetoprim as did the final reaction with sodium methoxide and the condensation with guanidine. On a scale of 5 mmol of barium carbonate (6.04 mCi/mmol, 30.2 mCi total), overall radiochemical yield of product (6) with specific activity of about 20 μCi/mg was 20%. Repeating the sequence on the same 5-6 mmol scale with highly enriched carbon-14 dioxide (55-60 mCi/mmol), provides the same overall radiochemical yield of about 20% but the product has specific activity which is close to that of the starting carbon dioxide as expected.

EXPERIMENTAL

General. Radioactivity was measured by the liquid scintillation technique using a Packard Tricarb Model 2003 spectrometer. All solvents were distilled prior to use. Tetrahydrofuran was distilled from benzophenone ketyl. Radiochemical purity was determined on thin layer chromatograms with a Packard Model 7201 Radiochromatogram Scanner System or an LB 2832 Berthold TLC Linear Analyzer System.

4,5-Dimethoxy-2-methylbenzoic-7-^{14}C acid (2) - A Grignard reagent was prepared (under N_2) from 363 mg of iodine activated magnesium turnings and 2 mL of a tetrahydrofuran solution containing 346 mg (1.5 mmol) of 2-bromo-4,5-dimethoxytoluene (1).[2] The reaction was initiated by the addition of a drop of 1,2-dibromoethane after which a further portion of 18 mL of the THF solution of 1 (15 mmol total of 1) was added at a rate that maintained gentle reflux. When the addition was completed, reflux was continued until the magnesium had disappeared. One half of the resulting solution containing 7.5 mmol of Grignard reagent was transferred by pipette into a carbonation apparatus and after typical treatment[3] carbonated with carbon-14 dioxide obtained from 989 mg (5 mmol) of barium carbonate-^{14}C having specific activity of 6.04 mCi/mmol. The carbonation was carried out with stirring at -80°C for 20 min followed by 20 min at -25°C. The system was then filled with dry nitrogen and the addition of 10 mL of 6N HCl effectively quenched the reaction. The mixture was concentrated *in vacuo* to remove THF and then extracted with three 25 mL portions of ether. These were combined and extracted with five 15 mL portions of 10% aqueous sodium carbonate solution which in turn were combined, acidified to pH 1 with 6N HCl and again extracted with three 25 mL portions of ether. These were combined and dried over anhydrous magnesium sulfate, filtered and concentrated to a crystalline residue of 831 mg (84%) of 4,5-dimethoxy-2-methylbenzoic-7-^{14}C acid (2). The product corresponded to an authentic

sample of nonlabeled 2 by TLC on silica gel (chloroform, ethyl acetate, methanol, ammonia; 30:30:30:1) and was greater than 98% radiochemically pure.

4,5-Dimethoxy-2-methylbenzyl-7-^{14}C alcohol (3) - The sample of 2 obtained above (831 mg, 4.2 mmol) in the minimum amount of methanol needed for solution was slowly added to an ice-cold solution of diazomethane in ether prepared from 2.146 g (10 mmol) of N-methyl-N-nitroso-p-toluenesulfonamide and distilled with 30 mL of ether. After stirring at 0° for 10 min, the mixture was concentrated in vacuo to an oil which was dissolved in 10 mL of fresh ether. This was filtered, combined with an ether extract of the filter cake and added to a slurry of 355 mg (9.46 mmol) of lithium aluminum hydride in 10 mL of ether. The resulting mixture was stirred for 45 min at room temperature and then 4 mL of saturated aqueous ammonium chloride solution was added. This mixture was filtered through Celite and magnesium sulfate and the filtrate concentrated in vacuo to 758 mg (98%) of oil.

4,5-Dimethoxy-2-methylbenzaldehyde-7-^{14}C (4) - The alcohol (3) obtained above, 758 mg (4.16 mmol) dissolved in 20 mL of benzene, was stirred with 5.3 g (60 mmol) of activated manganese dioxide for 3.25 h at room temperature. The mixture was filtered and the filtrate, after drying over anhydrous magnesium sulfate, was concentrated in vacuo to 699 mg of residual oil. By TLC on silica gel, chloroform elution, only aldehyde 4 was present and was 98% radiochemically pure.

4,5-Dimethoxy-2-methyl-2'-methoxymethylcinnamonitrile-3'-^{14}C (5) - All of the aldehyde (4) obtained above (3.88 mmol) was combined with 455 mg (5.34 mmol) of freshly distilled 2-methoxypropionitrile and 10 mL of methanolic sodium methylate solution (4.8 mmol of $NaOCH_3$) and heated under reflux for 22 h. An additional 260 mg of freshly distilled 2-methoxypropionitrile was added and reflux was again maintained for 22 h.

After this time, the solution was cooled, added to 20 mL of water and extracted with five 20 mL portions of benzene. These were combined, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo to 766 mg of residual oil corresponding to 5 which was used without further purification.

2,4-Diamino-5-(4,5-dimethoxy-2-methylbenzyl-7-^{14}C)-pyrimidine (6) - The adduct 5 obtained above, 766 mg (3.1 mmol) and 8.8 mL of methanolic sodium methylate solution (containing 7.75 matom sodium) were heated under mild reflux for 2.25 h. Methanol was then distilled out while the mixture was kept at 95-100° for an additional 3 h when 15 mL of methanolic guanidine (prepared by the addition of 12.4 mmol guanidine hydrochloride to 12.4 mmol of sodium methoxide and separating the precipitated sodium chloride) was added. This mixture was heated under reflux for 16 h when again, methanol was distilled out while maintaining the mixture at 95-100°C for an additional 2.25 h. After cooling, 30 mL of chloroform was added to effect a solution which was extracted with five 30 mL portions of water. These were combined and extracted with three 30 mL portions of chloroform. All of the chloroform solutions were combined and extracted with five 25 mL portions of 10% acetic acid which were combined and alkalinized with 50% sodium hydroxide solution to precipitate the crude product which was 90-95% radiochemically pure by TLC on silica gel (benzene, ethyl acetate, methanol, ammonia solution; 50:50:7.5:1 or ethyl acetate, methanol, ammonia solution; 80:20:1 elution). The product was purified to greater than 99% radiochemical purity by chromatography over 8 g of silica gel (E. Merck No. 7734) packed in ethyl acetate, methanol and ammonia solution, 80:20:1 and eluting the product with this mixture. A total of 327 mg (1.19 mmol) 6.12 mCi of 6 was obtained. Radiochemical yield from barium carbonate-^{14}C was 20%.

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