Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXIV, No. 6

SYNTHESIS OF TRITIATED ß-(S-BENZYLMERCAPTO)-ß,ß-CYCLOPENTAMETHYLENE PROPIONIC ACID

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ß-(S-benzylmercapto)-ß,ß-cyclopentamethylene propionic acid has been tritium labeled in the cyclohexyl ring. A key feature of the synthesis is the reduction of phenolwith tritium gas over catalyst giving tritiated cyclohexanone. A Wadsworth-Emmons reaction followed by esterase hydrolysis of the resulting ester and Michael addition of p-methylbenzylmercaptan afford the title compound.

Key Words: Tritium reduction, cyclohexanone, esterase hydrolysis.

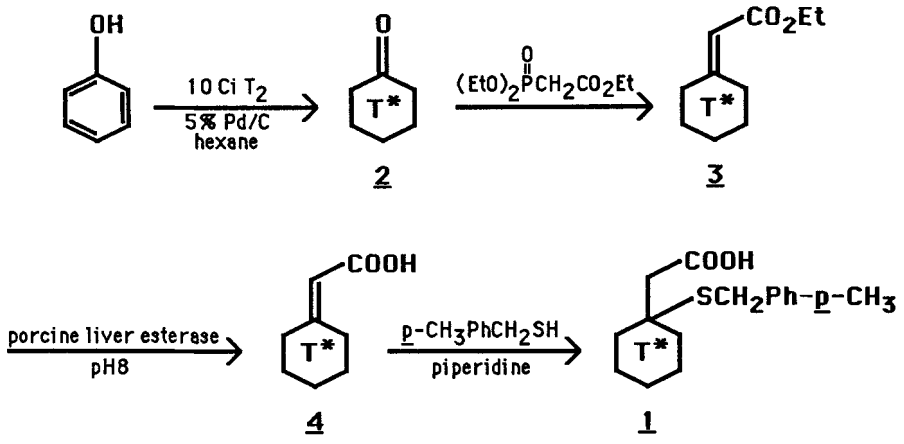
ß-(S-benzylmercapto)-ß,ß-cyclopentamethylene propionic acid (S-p-methylbenzyl-PMP) is a vital component in the synthesis of several arginine vasopressin antagonists (1) including the potent cyclic octapeptide SK&F 101926 (2). Since this is an unnatural component of the peptide, its metabolism and ultimate fate in vivo were of interest. Such metabolism studies required the synthesis of high specific activity tritiated S-p-methylbenzyl-PMP 1. The synthesis (Scheme I) is accomplished by reducing phenol with tritium gas to tritiated

0362-4803/87/060689–06$05.00

Received August 20, 1986

cyclohexanone in hexane solution over 5% Pd/C. Though this reduction

Scheme I



T* denotes general tritium labeling in the cyclohexyl ring.

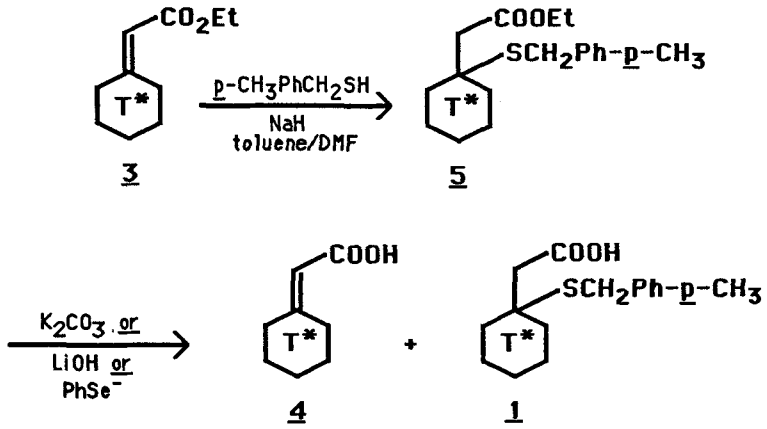
has been previously reported for unlabeled cyclohexanone (3), this is the first reported synthesis of tritiated cyclohexanone obtained by reduction of phenol. Analysis by GC indicates that the only product is cyclohexanone (plus some unreacted phenol). The labeled cyclohexanone (2) is treated with triethylphosphonoacetate giving tritiated ethyl cyclohexylidene acetate 3 (4) in 27% overall radiochemical yield from tritium gas after HPLC purification. The ester is hydrolyzed rapidly and cleanly with porcine liver esterase type I (pH 8). The acid (4) thus obtained is directly treated with p-methylbenzylmercaptan in refluxing piperidine for 18 hours. Purification by HPLC affords S-p-methylbenzyl-[^{3}H]PMP (1).

Deuterium model studies and analysis by mass spectroscopy on PMP ester 3 indicate that 3.44 $\pm$ .34 deuterium atoms are incorporated per molecule. Thus, a specific activity of 99.8 Ci/mmol is predicted; a specific activity of 96.9 $\pm$ 2.3 Ci/mmol is actually obtained.

The reaction conditions required for this synthesis differ quite markedly from the synthesis of unlabeled S-p-methylbenzyl-PMP (4)

where Michael addition of the mercaptan, using NaH, is carried out prior to aqueous potassium carbonate hydrolysis of the ester (5). On the small scale which tritiation necessitates, such a reaction sequence (Scheme II) leads uniformly to the production of cyclopentamethylene-propenoic acid 4 as the major product (80-100%). Evidently, base-catalyzed elimination of mercaptan from S-p-methylbenzyl-PMP ester 5 is much more rapid than hydrolysis, since exposure of S-p-methylbenzyl-PMP (1) itself to these reaction conditions does not lead to the formation

Scheme II



of this compound. Other chemical methods (hydrolysis with NaOH, Li_2CO_3, NaSEt or BBr_3) all fail. Moderate success is obtained with LiOH hydrolysis (25-30% S-p-methylbenzyl-PMP 1/70-75% propenoic acid 4) and phenyl selenide hydrolysis (6) (67% S-p-methylbenzyl-PMP 1/33% propenoic acid 4). However, none of these reagents works as well as the esterase which, on a tracer level, consistently gives the desired hydrolysis product. Unfortunately, on a large scale radioactive synthesis, sulfide impurities in the ester 5 inhibit enzyme activity. This problem is alleviated by reversing the last two steps and carrying out the esterase hydrolysis on the easily purifiable PMP ester 3.

EXPERIMENTAL SECTION

Porcine Liver Esterase Type I (pH 8) was purchased from Sigma. Triethylphosphonoacetate was purchased from Aldrich. Tritium gas was purchased from New England Nuclear. GC analysis was carried out on a 5% carbowax 20M column on 100/120 mesh Supelcoport (2mm ID x 2M) at 60° for 13 min, then 10 /min to 150°. Deuterium incorporation was determined using a Finnegan 1020 mass spectrometer. Phenol was distilled prior to use and a saturated solution prepared by adding to dry hexane (distilled from sodium). Toluene was dried via distillation from sodium. P-Methylbenzylmercaptan was purchased from Fairfield Chemicals and was distilled prior to use. Piperidine was distilled and stored over 4Å molecular sieves. Compound identities were confirmed by coinjection and subsequent coelution with with authentic unlabeled compounds (both GC and HPLC).

[^{3}H]Cyclohexanone (2). In a flame-dried conical flask with side arm was placed 6.4 mg dry phenol (68 mmol) in 0.246 mL dry hexane . To this was added 6.4 mg 5% Pd on C (Engelhard). The flask was placed on a Toepler pump, cooled in liquid nitrogen and 10 Ci tritium gas introduced. The mixture was warmed to room temperature and the reaction stirred 24 hrs. GC analysis indicated cyclohexanone as the only product. The mixture was again cooled in liquid nitrogen and the remaining tritium gas transfered to a flask containing PtO_2 . The product was used directly in the next reaction without any further purification or characterization.

[^{3}H]Ethyl Cyclohexylidene Acetate (3). [^{3}H]Ethyl cyclohexylidene acetate was prepared according to the method of Wadsworth and Emmons (4) except that toluene was substituted for benzene and the reaction mixture was stirred at room temperature for 24 hrs. GC analysis showed the reaction to be complete. The product was purified by preparative HPLC (LiChrosorb Si60, 10mm x 25cm, 75:25 hexane/CH_2Cl_2 , 3 mL/min, UV at 220 nm). The product was taken to dryness in vacuo and reconstituted in absolute EtOH giving 2.75 Ci (27.5% radiochemical yield

from phenol) at a radiochemical purity of 99.3% and a specific activity of 96.9 Ci/mmol (both determined by HPLC on a LiChrosorb Si60 column, 4.6mm x 25cm, 99:1 hexane/0.67% trifluoroacetic acid in ether, 1.5 mL/min, UV at 220 nm with radioactivity monitored with a Radiomatic Flo-1 HPLC radioactivity detector).

[^{3}H]-ß,ß-Cyclopentamethylenepropenoic Acid (4). A 180 mCi portion of [^{3}H]ethyl cyclohexylidene acetate (3) in 50 mL EtOH was added to 1 mL pH 8 phosphate buffer. To this was added 100 mL porcine liver esterase (Type I, pH 8). The mixture was stirred 4 hrs at room temperature, quenched with 6N HCl and extracted with ether. The organic layer was dried (Na_2SO_4). The solvent was removed under a stream of dry argon and the product taken up in 5 mL EtOH. HPLC analysis shows the crude product (171 mCi) to be 85% radiochemically pure (81% radiochemical yield) with the remaining portion being unreacted ester (LiChrosorb DIOL column,10mm x 25cm, 85:15 hexane/0.67% trifluoroacetic acid in ether, 3 mL/min, UV at 220 nm). The product was used directly in the next reaction without any further purification or characterization.

[^{3}H]-ß-(S-Benzylmercapto)-ß,ß-Cyclopentamethylene Propionic Acid (1). Propenoic acid 4 (145 mCi, 1.5 mmol) in 5 mL EtOH was taken to dryness in vacuo. This was diluted with 0.55 mg (3.9 mmol) unlabeled 4. To this was added 3.66 mg p-methylbenzylmercaptan (26.5 mmol) and 1 mL piperidine. The reaction mixture was heated to 125° in a sealed reactivial 24 hrs. The reaction was quenched with 6N HCl, extracted with ether and the organic layer dried (Na_2SO_4). HPLC analysis (LiChrosorb DIOL column, 10mm x 25cm, 85:15 hexane/0.67% trifluoroacetic acid in ether, 3 mL/min, UV at 220nm) indicated that 80% of the mixture was the desired [^{3}H]PMP. The remaining 20% was a mixture of propenoic acid 4 and its double bond isomer (endocyclic double bond isomer, identified by coinjection). The material was purified by preparative HPLC using the above system. The purified product was lyophilized and taken up in

absolute EtOH giving 86 mCi (50% radiochemical yield) of [^{3}H]PMP (1). Radiochemical purity of the final product was greater than 92% as determined by HPLC; this was of sufficient purity to be used directly in our solid phase peptide syntheses (7).

ACKNOWLEDGEMENTS

We would like to thank Dr. Wil Kokke and Mr. Sid Levinson for their assistance in the GC analysis of products 2 and 3. Further thanks go to Dr. Kokke for his work in determining the deuterium content of 3 in our model studies. We would also like to thank Dr. Nelson Yim for his suggestions on the addition of the mercaptan to unsaturated acid 4.

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