A NEW SYNTHESIS OF LEVULINIC-4-^{14}C ACID - A PRECURSOR FOR THE PREPARATION OF LABELED INDOMETHACIN

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SUMMARY

A new procedure utilizing carbon-^{14}C dioxide for the preparation of levulinic-4-^{14}C acid has been developed. This acid is a key intermediate for the synthesis of indomethacin-2-^{14}C.

Key Words: 1-(p-Chlorobenzoyl)-5-methoxy-2-^{14}C-indole-3-acetic acid, Indomethacin-2-^{14}C, Levulinic-4-^{14}C acid

DISCUSSION

Since reports (1) that indomethacin possesses considerable antiinflammatory, antipyretic, and analgesic activities, carbon-14 labeled material has been needed for use in metabolic and other tracer studies (2). This laboratory has supplied the labeled material needed for such studies, and continues to prepare the labeled drug to meet new demands.

A suitable tracer for metabolic and pharmacokinetic studies should be labeled such that no unexpected label loss or transfer can occur to complicate the interpretation of results. Indomethacin labeled with carbon-14 at a ring position was thought to meet this criterion satisfactorily. A ready approach to such a labeling pattern was found in the useful procedure for preparing indomethacin in which N-(p-chlorobenzoyl)-4-methoxyphenylhydrazine is caused to react with levulinic acid (Scheme I). An accessible precursor to furnish ring labeled (carbon-2 of the five membered indole ring) tracer using this process is levulinic-4-^{14}C acid. A description of an adaptation of the practical levulinic acid synthesis of Mentzer and Billet (3) to the preparation of

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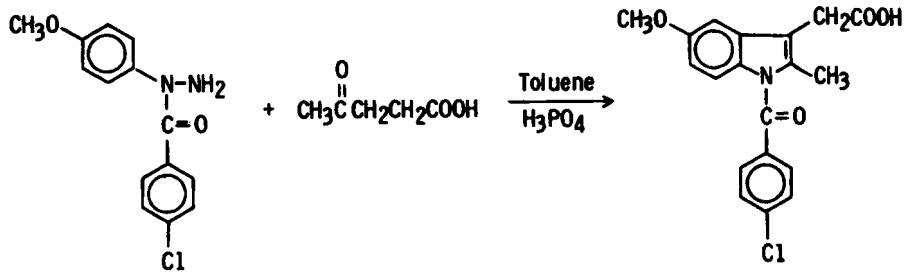
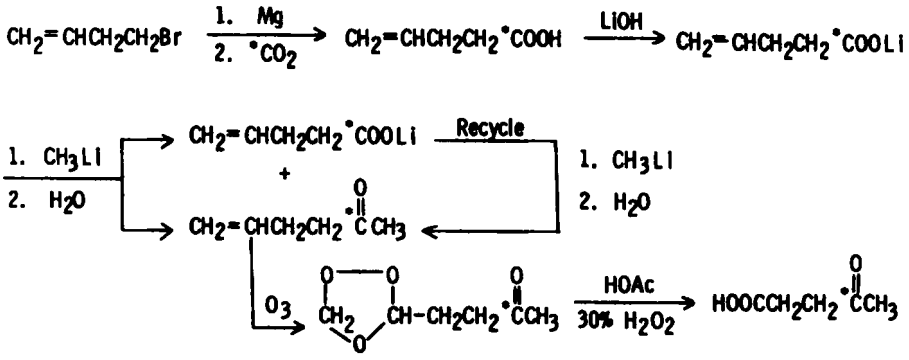


Scheme I

levulinic-4-^{14}C acid has been published (4). Our laboratory has also used this route for preparing levulinic-4-^{14}C acid to be used as a label carrier for the synthesis of indomethacin and other related indoleacetic acids. This conventional route to levulinic-4-^{14}C acid is based on ethyl acetoacetate-3-^{14}C, which in turn is derived from acetate-1-^{14}C, using a procedure first described by Breslow, Baumgarten, and Hauser (5). The entire improved sequence from acetate-1-^{14}C to levulinic-4-^{14}C acid, with an overall yield of 25 %, is detailed in the recently published article already cited (4). In our several previous syntheses of indomethacin and analogues via levulinic-4-^{14}C acid ethyl acetaacetate-3-^{14}C from commercial sources was employed, rather than to go through the extra and tedious steps from acetate-1-^{14}C. Labeled acetoacetic ester in substantial quantity proved to be expensive, and as a custon preparation to order, was often of marginal purity, and usually occasioned delays in supply.

These difficulties led us to seek a new method for preparing ^{14}C-labeled levulinic acid from a readily available radioactive starting material. A simple, efficient procedure which generated levulinic-4-^{14}C acid in 63 % yield from carbon-^{14}C dioxide has now been developed.

Our new procedure, as illustrated in Scheme II, introduces the label via carbonation of bromo-3-butenylmagnesium with carbon-^{14}C dioxide (6). The 4-pentenoic-1^{14}C acid so generated was isolated as the lithium salt by partitioning from ether into an aqueous solution containing just sufficient



Scheme II

lithium hydroxide to form the salt (final pH: 9.85). After removal of bulk water and vacuum drying (residual water by Karl Fischer analysis < 0.2 %), the lithium 4-pentenoate-1-^{14}C was suspended in dry ether and 1.2 equivalents of methyllithium was added to it. This treatment led to a mixture containing approximately equal amounts of 5-hexen-2-one-2-^{14}C and unreacted lithium carboxylate. The unreacted lithium pentenoate was again partitioned into water, isolated, and dried; and was again treated with methyllithium (1.2 equivalents) to give, again, about 50 % conversion to methyl ketone. The combined products from these two cycles totaled 34.8 mCi. Ozonolysis of this material at -78° yielded a crude ozonide which upon treatment with hydrogen peroxide in glacial acetic acid gave levulinic-4-^{14}C acid. Removal of the solvents by lyophilization gave 31.8 mCi of levulinic acid suitable for use in the synthesis of indomethacin. The overall radiochemical yield from carbon-^{14}C dioxide was 63.6 %.

EXPERIMENTAL

Analytical TLC was carried out on 5x20 cm glass plates precoated with silica gel 60 F-254 (E. Merck, Darmstadt, Germany). Radioactive zones were located with a Varian Aerograph/Berthold Model LB2722 scanner. Radioactivity was determined with a Packard Tri-Carb Model 3320 liquid scintillation spectrometer, using 0.4 % Omnifluor® in toluene/ethanol (7:3) as scintillator medium. Purity and specific activity of the starting carbon-^{14}C dioxide was taken as stated by the supplier.

4-Pentenoic-1-^{14}C Acid, Lithium Salt--To a Grignard solution prepared in the usual manner from 121.6 mg (5.0 mmoles) of magnesium turnings and 742.5 mg (5.5 mmoles) of freshly distilled 4-bromo-1-butene in 30 ml of ether was added, at -195° C by vacuum transfer, 42.3 mg (0.92 mmole, 50.0 mCi) of carbon-^{14}C dioxide (Amersham/Searle, Batch # 54). The reaction mixture was warmed to -30° C and aged for 3/4 hour, then was quenched at that temperature with 10 ml of water. The temperature was allowed to rise to 25° C and 2.0 ml of 2.5 N sodium hydroxide was added. After vigorous stirring, the ether solution (not radioactive) was removed, the aqueous phase was made acidic by the addition of 18 N sulfuric acid, and the product was extracted into 5x25 ml portions of ether. The combined ether extracts, dried over anhydrous magnesium sulfate, filtered, and diluted to 250 ml with ether, showed 47.4 mCi of product by scintillation counting. This ether solution was layered with 10 ml of water, and dilute aqueous lithium hydroxide solution was added with vigorous stirring to a final pH of 9.85. The aqueous layer was separated, 212 mg (2.0 mmoles) of non-radioactive carrier salt was added, and the aqueous solution (pH 9.9) was evaporated *in vacuo* to leave a residual solid which after drying at 100°/0.5 mm weighed 336 mg (47.4 mCi).

5-Hexene-2-one-2-^{14}C--To 336 mg (3.0 mmoles, 47.4 mCi) of dried 4-pentenoic--1-^{14}C acid lithium salt in a 100 ml, 1-necked, RB flask was added 25 ml of dry (CaH_2) ether. The mixture was stirred with a magnetic bar until the solid was well dispersed, and then 1.8 ml (3.6 mmoles) of methyllithium solution was added in one portion. The flask was stoppered and the contents stirred at 25° C for 4 hours. The reaction mixture was quenched with 5 ml of water and the aqueous solution was withdrawn and concentrated to dryness to yield 170 mg of unreacted lithium carboxylate-^{14}C. This solid was treated as above with another portion of methyllithium (1.0 ml, 2.0 mmoles). The ether layers from the two cycles were combined and dried over anhydrous magnesium sulfate, filtered, and ether was removed by atmospheric pressure distillation through a helices-packed column to a final volume of 2-3 ml.

This solution was diluted with 40 ml of methylene chloride and reconcentrated to a similar volume. This concentrate, diluted again with 50 ml of methylene chloride and analyzed by GLC (3 % OV-17, 6 ft. column, 30° C) and by scintillation counting, showed 34.8 mCi of pure 5-hexene-2-one-2-^{14}C. In order to insure complete removal of ether, the solution was concentrated once again, and this concentrate (5 ml) was used in the next, the ozonolysis step.

Levulinic-4-^{14}C Acid--A solution of 5-hexene-2-one-2-^{14}C (34.8 mCi, 2.31 mmoles) in 5 ml of methylene chloride was treated at -78° C with ozone until the reaction mixture turned blue (20 minutes) and then aged for 5 minutes longer. Solvent was removed by distillation (reduced pressure) at 0-5° C until a residual ozonide (CAUTION - the stability of this potentially explosive intermediate was not determined), which weighed 400 mg, was obtained. To this concentrate was then added 2.5 ml of glacial acetic acid and 0.5 ml of 30 % hydrogen peroxide. This mixture was heated to 75° c over a period of 2 hours, then heated to reflux over an additional ½ hour. The reaction mixture was maintained at reflux until GLC analysis (3 % OV-17, 6 ft. column, 120° C) showed essentially pure product was present (105 minutes). Removal of most of the acetic acid and water by lyophilization gave a concentrate containing 31.6 mCi of levulinic-4-^{14}C acid suitable for indomethacin formation.

1-(p-Chlorobenzoyl)-5-methoxy-2-methyl-2-^{14}C-indole-3-acetic Acid, Indomethacin-2-^{14}C--A mixture of 301.3 mg (1.09 mmoles) of α-(p-chlorobenzoyl)-p-methoxyphenylhydrazine, 675.8 mg of a concentrate which contained 1.09 mmoles of levulinic-4-^{14}C acid (as determined from the total and specific radioactivity), 15.8 mCi, 0.32 ml of 85 % phosphoric acid, and 10 ml of toluene was heated under N_2 and azeotropic conditions until water evolution ceased (about 2 hrs.). The hot solution was decanted from an insoluble tar, diluted with additional toluene, and then extracted with 3x10 ml of water at 80-90° C. The toluene layer was dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated in vacuo to a small volume to yield 405.5 mg (12.9 mCi) of crude (90 % by TLC analysis; silica, CCl_4:HOAc, 9:1) indomethacin-2-^{14}C.

Tracer quality material was obtained by slurrying the crude product in a mixture of methanol and water (2:1), followed by recrystallization from toluene.

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