SYNTHESIS OF ^{14}C-LABELLED (+)-LIMONENE.

K. Noda, T. Matsuda and Y. Ishikura.
Research Laboratories, Hisamitsu Pharmaceutical Co.,
Inc. Tosu, Saga, Japan.

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SUMMARY

^{14}C-labelled (+)-limonene was conveniently prepared by the Wittig reaction of (+)-1-methyl-4-acetylcyclohexene with methyl-^{14}C-triphenylphosphonium iodide.

INTRODUCTION

It has been recently discovered in these laboratories that (+)-limonene, which has been widely used in perfume and flavor compositions, is an effective solubilizer for gallstones, especially cholesterol stones found in cholelithiasis. As a matter of course, studies on the metabolic fate of (+)-limonene in mammals including human have required the preparation of specifically labelled (+)-limonene. Present paper deals with the synthesis of (+)-limonene-9-^{14}C using the Wittig reaction.

Vig, et. al.(1,2) have already reported that (±)-limonene has been synthesized by reacting the Wittig reagent with (±)-1-methyl-4-acetylcyclohexene obtained from the starting ethyl 4-(2-ethoxycarbonylethyl)-5,5-ethylenedioxyhexanoate via five steps. It is, however, extremely difficult to prepare the optically active (+)-limonene (Ia) by this method. If (+)-1-methyl-4-acetylcyclohexene (IV) with the same configuration as that of (+)-limonene (Ia) could be obtained, then (+)-limonene could be synthesized by applying the Wittig reaction as shown in Chart 2. Consequently, our attempts have been made to prepare (+)-1-methyl-4-acetylcyclohexene (IV) from (+)-limonene (Ia) using a method similar to that reported by Aratani(3) and Ogata(4), in which the optical activity of the compound (IV) might be retained.

RESULTS

As shown in Chart 1, 1-methyl-4-(1-methyl-1-hydroxy-2-acetoxy)ethylcyclohexene (II) was obtained by treating (+)-limonene (Ia) with lead tetroxide in acetic anhydride and acetic acid. The monoacetate (II) thus obtained was deacetylated with 0.5 N alcoholic potassium hydroxide solution, and further oxidized with lead tetraacetate to yield the desired compound (IV), (+)-1-methyl-4-acetylcyclohexene, which was identical with (±)-1-methyl-4-acetylcyclohexene separately prepared by Vig's method with the exception of the optical activity.

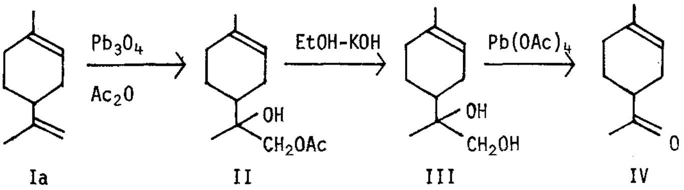

Chart 1

Since it was successful to prepare the starting material for (+)-limonene-9-^{14}C (Ib), further attempts were made to introduce carbon-14 to (+)-limonene (Ia), as shown in Chart 2. Prior to the Wittig reaction, the synthesis of Wittig reagent, methyl-^{14}C-triphenylphosphonium iodide (V) was carried out under vacuum and the reagent was prepared in a high yield without any difficulties. The compound thus obtained was ready for the Wittig reaction of the last step.

As shown in Chart 2, carbon-14 was introduced to the methylene carbon of the side chain at the 9 position, and hence the optical activity of the object compound was to be retained completely. Moreover, the (+)-limonene-9-^{14}C (Ib) thus obtained was actually identical with the natural (+)-limonene (Ia) with regard to the various physical constants and the data of instrumental analysis. No significant difference was observed between the radiochemical yield (47 %) and chemical yield (44 %), both of which were based on methyl-^{14}C-triphenylphosphonium iodide, a reagent which was used in excess.

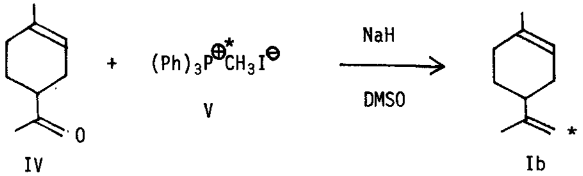

Chart 2

EXPERIMENTAL

Radioactivity measurements - - The radioactivity in each sample was quantified in a liquid scintillation spectrometer (Packard Tri-Carb, 3375) using the scintillator mixture (POP 5 g, dimethyl-POPOP 0.3 g and toluene 1000 ml).

(+)-1-Methyl-4-acetylcyclohexene (IV) - - To 1200 ml of acetic acid and 420 ml of acetic anhydride was added 90 g (0.66 mol) of (+)-limonene (Ia). To the solution was slowly added 450 g (0.66 mol) of lead tetroxide over a period of 3 hours with stirring, keeping the temperature of solution at 60°. Stirring was further continued for one hour after adding lead tetroxide. To the mixture was added 10 l of water and the mixture was neutralized with sodium carbonate and then extracted with benzene. The extract was washed with diluted sodium carbonate solution, further washed with water and dried over anhydrous sodium sulfate. The benzene was distilled off to give 120 g of the oily product mainly consisting of 1-methyl-4-(1-methyl-1-hydroxy-2-acetoxy)ethylcyclohexene (II). To the oily product was added 5 l of 0.5 N alcoholic potassium hydroxide solution. The solution was refluxed for one hour and extracted with ethyl acetate after adding 3 l of water. The extract was concentrated by distillation to less than one-half of the original volume, washed with water and dried. The ethyl acetate was distilled off to give the faintly yellow, viscous liquid mainly consisting of 1-methyl-4-(1-methyl-1,2-dihydroxy)ethylcyclohexene (III). The liquid was then dissolved in 500 ml of benzene. To the solution was slowly added 80 g (0.18 mol) of lead tetraacetate under reflux in nitrogen atmosphere, and the mixture was refluxed for one hour with stirring. After cooling, the crystals were produced and filtered off. The filtrate was dried and the benzene was distilled off to give the residue. The further distillation of the residue under reduced pressure afforded 11.48 g (0.08 mol) of (+)-1-methyl-4-acetylcyclohexene (IV), bp 70°/4 torr as a colorless, fragrant liquid: n_d^{25} 1.4710; d_4^{25} 0.9380; $[\alpha]_D^{20}$ +88.1°; semicarbazone (from ethanol) mp 160 - 1°; oxime (from ethanol/water) mp 50 - 1°; ir 1711 cm^{-1}(C=O), 801 cm^{-1}(C=C-H); mass spectrum m/e 138(M^+).

Methyl-^{14}C-triphenylphosphonium iodide (V) - - To the solution of 2.50 g (9.5 mmol) of triphenylphosphine in 3 ml of anhydrous benzene was slowly added 1.11 g (7.8 mmol) of methyl-^{14}C iodide (10 mCi, 1.28 mCi/mmol) and 1 ml of anhydrous benzene in vacuo under cooling in a dry ice-acetone bath. The mixture was allowed to stand overnight at ordinary pressure and temperature. The crystals were produced, filtered and washed thoroughly with anhydrous benzene. Drying at 100° under reduced pressure (1 torr) for 4 hours afforded 2.70 g (6.7 mmol) of methyl-^{14}C-triphenylphosphonium iodide (V): Chemical yield 85 %. To 1.4 mg of methyl-^{14}C-triphenylphosphonium iodide in 1 ml of acetone added 15 ml of the scintillator mixture. The radioactivity was counted for the resulting solution : radiochemical yield 9.78 mCi (98 %).

Apparatus for synthesis of methyl-^{14}C-triphenylphosphonium iodide (V) - - The synthesis of methyl-^{14}C-triphenylphosphonium iodide was carried out in the reaction device consisting of five flasks, eight stopcocks and a manometer, as shown in Fig. 1. Both in the flask A and B was placed each 5 mCi of methyl-^{14}C iodide (32.0 mCi/mmol; made by Daiichi Kagaku Co., Ltd.). In the flask C, D and E were placed methyliodide, 2.5 g of triphenylphosphine in 3 ml of anhydrous benzene and 1 ml of anhydrous benzene respectively. After each flask was cooled in each dry ice-acetone bath, the device was connected to a vacuum line and all the stopcocks were closed except the stopcock g and h. Methyl-^{14}C iodide in the flask A and B were transferred into the flask D. Transferring every reagent was conducted as follows: the stopcocks

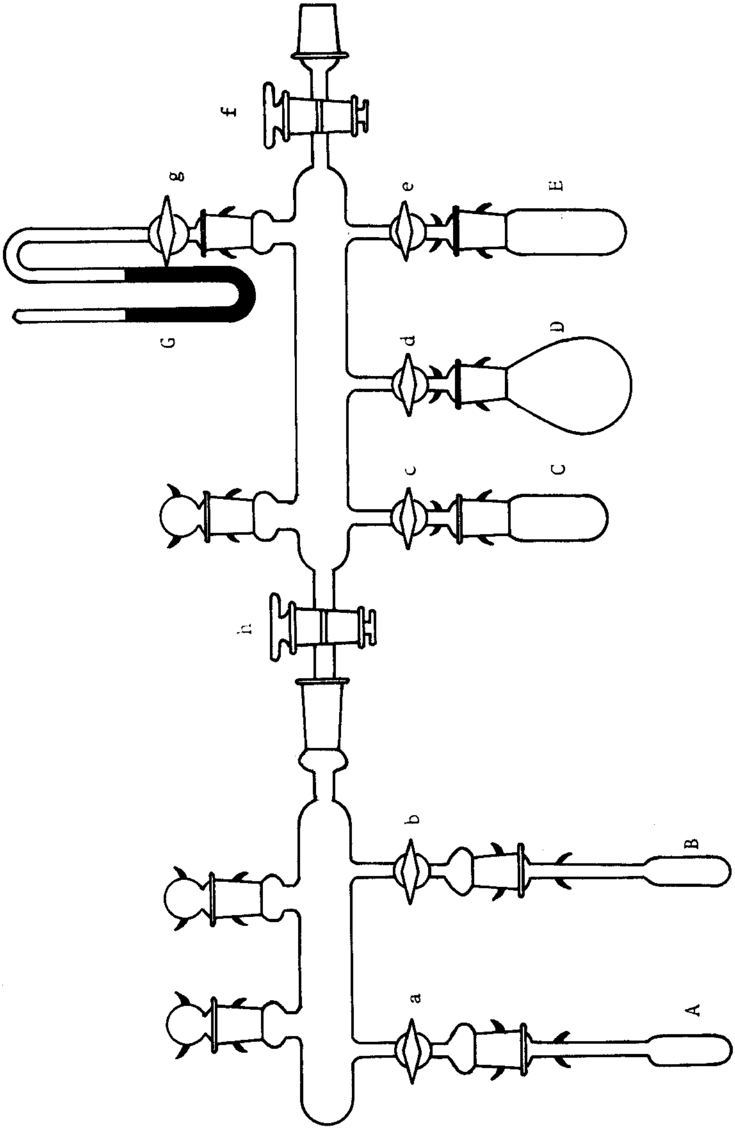

Fig.1. Apparatus for synthesis of methyl-^{14}C-triphenylphosphonium iodide

of both the supplying and the receiving flask were opened, and only the former flask was allowed to warm gradually to about 60°, after removing the bath. Methyliodide in the flask C was transferred into the flask A and B for washing and further transferred into the flask D. Similarly, benzene in the flask E was transferred into the flask A and B for washing and further transferred into the flask D. The device was brought gradually to the ordinary pressure, stoppered tightly and allowed to stand overnight at a room temperature.

(+)-Limonene-9-^{14}C (Ia) - - To the solution of 2.65 g (6.6 mmol) of methyl-^{14}C-triphenylphosphonium iodide (V) (9.60 mCi) in 7.2 ml of anhydrous dimethylsulfoxide was added 0.36 g (15.0 mmol) of sodium hydride with stirring under cooling, introducing a current of nitrogen, and further added 0.45 g (3.3 mmol) of (+)-1-methyl-4-acetylcyclohexene (IV) in 7 ml of anhydrous tetrahydrofuran. The mixture was heated at 50° for 2 hours and allowed to cool to room temperature. After cooling, the equal volume of water was added to the mixture to decompose the unreacted Wittig reagent. The mixture was extracted continuously for 20 hours with ether. The extract was dried and ether was then removed off to give the residue. The further distillation under reduced pressure afforded 0.39 g (2.9 mmol) of (+)-limonene-9-^{14}C (Ib), bp 55°/2 torr as a colorless, fragrant oil: n_d^{20} 1.4730; d_4^{20} 0.840; $[\alpha]_D^{20}$ +126.8°; mass spectrum m/e 136 (M^+, $C_{10}H_{16}$); chemical yield 44 %. To 100 ml was diluted 7.7 mg of (+)-limonene-9-^{14}C with acetone. To 1 ml of the acetone solution was added 15 ml of the scintillator mixture. The radioactivity was counted for the resulting solution: radiochemical yield 4.53 mCi (47 %); recovery of radioactivity 9.25 mCi (96 %).

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