SYNTHESIS OF RING ^{14}C-METHYL LABELED RETINYL ACETATE

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SUMMARY

A method for incorporation of a ^{14}C-label in the ring geminal methyl moiety of Vitamin A is described. The procedure involves mono methylation of 2,6-dimethylcyclohexanone with ^{14}C-methyl iodide as catalyzed by potassium hydride to afford 2-^{14}C, 2,6-trimethylcyclohexanone. Subsequent condensation with an 11-carbon acetylenic side chain, followed by reduction, acetylation and dehydration yielded ^{14}C-retinyl acetate. Final purification was achieved by preparative thin layer chromatography.

Key Words: ^{14}C-labeled Vitamin A, Mono Methylation, Potassium Hydride

DISCUSSION

Studies on the possible catabolism of Vitamin A in lung tissue as promoted by exposure to environmental factors required a synthesis of Vitamin A-^{14}C. A ^{14}C-label in the vicinity of the cyclohexyl terminus was desired so that the label would survive any extensive degradation of the polyene side chain. A synthesis of retinyl acetate with the ^{14}C-label in one of the geminal methyls on the cyclohexane ring was devised.

The method as shown in Scheme 1 is patterned after the procedure of Attenburrow et al.[1] The synthesis initiates from crotonylidene acetone (1) which was condensed with vinyl magnesium bromide to afford 3-methyl-1,4,6-octatrien-3-ol (2). This variation from the literature process[2]

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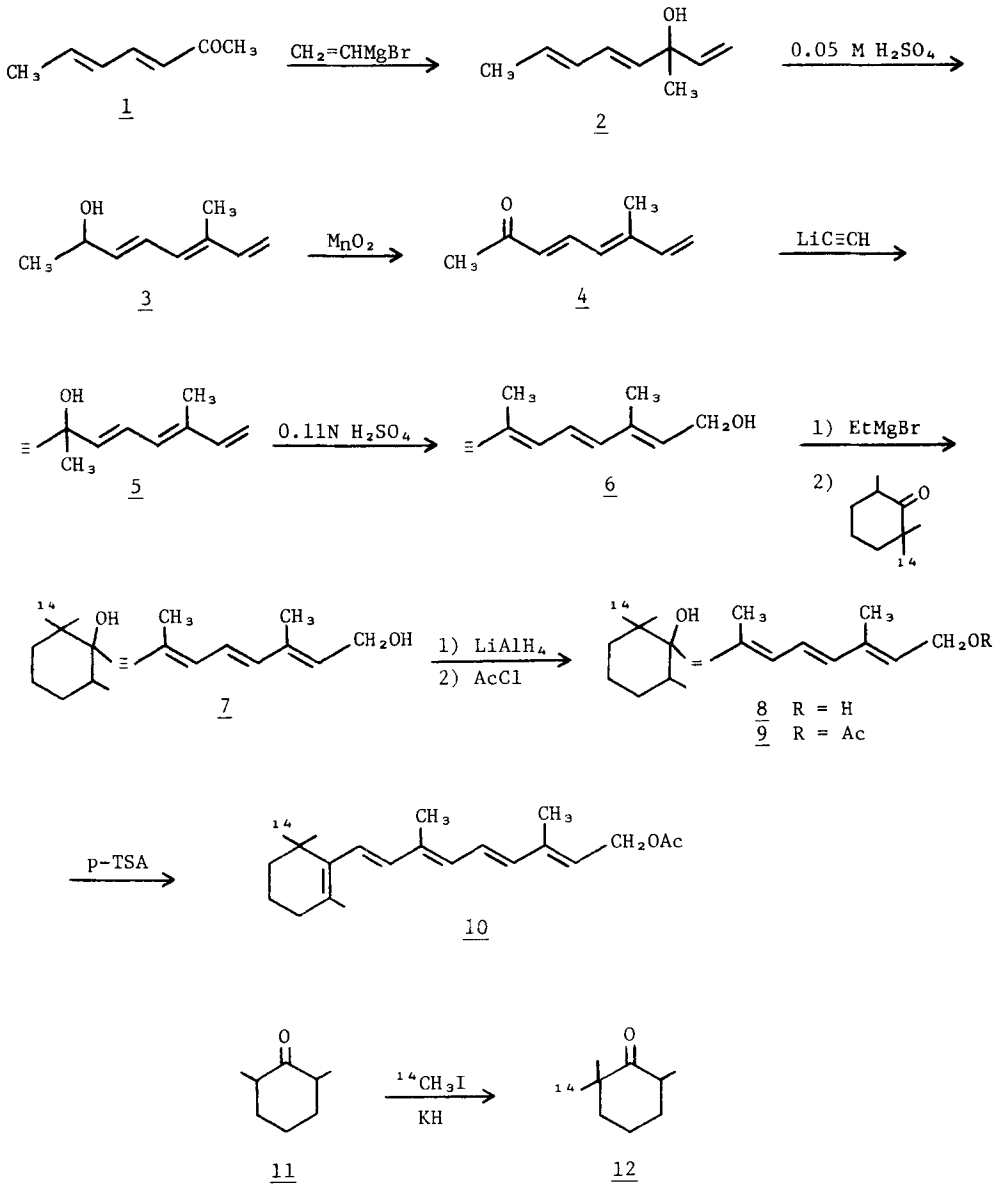

1 2 3 4 5 6 7 10 11 12

Scheme 1

circumvents the condensation of 1 with lithium acetylide and the potentially troublesome selective hydrogenation of the acetylenic function. Acid catalyzed rearrangement of the tertiary alcohol (2) to the secondary alcohol triene (3) is followed by manganese oxide oxidation of 3 to the trienone (4). Oppenauer oxidation[2] or MnO_2 in petroleum ether[1] have been reported to effect this transformation; however, only MnO_2 in chloroform was found to be satisfactory in our hands. Condensation of 4 with lithium acetylide gave the triene-yne-tertiary alcohol (5), which was readily rearranged to the primary alcohol (6) by mild acid treatment.

The ring ^{14}C-methyl was introduced by alkylation of 2,6-dimethylcyclohexanone (11) with ^{14}C-methyl iodide. Use of potassium hydride[3] caused rapid and complete ionization of the ketone so that a 90% yield of 2-^{14}C, 2,6-trimethylcyclohexanone (12) was achieved. Only 8% unreacted material and 2% tetramethyl ketone were observed as contaminants, thus avoiding a serious mixture of methylated ketones and the attendant tedious separation expected[1], especially on such a small scale labeled synthesis.

Condensation of the Grignard reagent derived from 6 (by exchange with ethyl magnesium bromide) with the ^{14}C-trimethylcyclohexanone gave the acetylenic carbinol (7). In the labeled runs the intermediate 7 was carried without further isolation of intermediates directly to retinyl acetate. It was considered beneficial to maintain these sensitive materials in a diluted state to minimize any photo or radiation induced polymerizations. The acetylenic triene (7) was reduced to the tetraene (8) with lithium aluminum hydride. Acetylation of the terminal hydroxyl

group (9) and acid catalyzed dehydration of the ring tertiary alcohol yielded crude retinyl acetate (10). Purification was accomplished by preparative thin layer chromatography. The isolated yield of retinyl acetate (5% from the trimethylcyclohexanone-^{14}C) was acceptable; however, much of the the losses appeared to be due to the inherent instability of the final compounds. Similar effects were noted in the unlabeled runs.

It is possible that the 2,6-dimethylcyclohexanone contaminant in 12 would have reacted in an analogous manner throughout the remainder of the sequence. This suggests contamination by a maximum of 8% of the ring desmethyl retinyl acetate, a material difficult to detect by most chromatographic or spectral methods. However, this contaminant may be further reduced, if not completely eliminated, by recrystallization of the intermediate acetylenic carbinol (7).

EXPERIMENTAL

3-Methyl-1,4,6-octatrien-3-ol (2)

Crotonylidene acetone (1) was prepared by the procedure of Attenburrow et al.[1] A solution of crotonylidene acetone (104.8 g, 0.95 mole) in ether (125 ml) was added over 2 hours to a solution of vinylmagnesium bromide (from 101.6 g, 0.95 mole of vinylbromide) in tetrahydrofuran (200 ml). A gentle reflux was maintained during the addition and was continued for another 30 minutes. The mixture was cooled and added slowly to ice cold saturated NH_4Cl (400 ml). The organic solution was separated, washed with saturated $NaHCO_3$, dried over $MgSO_4$ and evaporated in vacuo to leave a yellow oil (124 g). The residue was distilled at reduced pressure through a Vigreux column to afford 70.8 g (54%) of a colorless

liquid, bp 34-38° (0.3 mm); IR 3300 cm^{-1} (OH), 990 and 925 cm^{-1} ($-CH=CH_2$); NMR δ 6.40-4.90 (7H, m, olefins), 2.65 (1H, s, OH), 1.71 (3H, d, $CH_3CH=C$), 1.33 (3H, s, 3-CH_3). Cheeseman et al.[2] reported bp 65-67° (0.8 mm) for material prepared by a different route.

6-Methyl-3,5,7-octatrien-2-one (4)

Rearrangement of the tertiary alcohol (2) with 0.05 M sulfuric acid according to the procedure of Cheeseman et al.[2] afforded the octatrien-2-ol. (3). A mixture of 3 (5 g, 36 mmoles) and manganese dioxide (65 g) was stirred in chloroform (450 ml) for 18 hours under nitrogen. The mixture was filtered and the cake washed with chloroform. The filtrate was evaporated to dryness in vacuo to leave a yellow oil (4.8 g, 96%); IR 1680 cm^{-1} (C=O); NMR δ 6.6-5.2 (6H, m, olefin), 2.28 (3H, s, $CH_3C=O$), 2.03 (3H, s, $CH_3C=C$); UV (95% EtOH) λ_{max} 315 nm (ε = 23,000), reported 311 nm (31,000), see ref. 1.

3,7-Dimethyl-3,4,8-nonatrien-1-yn-3-ol (5)

To a stirred mixture of dry benzene (500 ml, saturated with acetylene) and lithium acetylide-ethylene diamine complex (19.7 g, 0.21 mole) was added a solution of 4 (3.0 g, 22.1 mmoles) in benzene (150 ml) over 1.5 hours. The resulting deep red solution was maintained at ambient temperature for 19 hours under an atmosphere of acetylene. The mixture was cooled in an ice bath and butylated hydroxytoluene (BHT) antioxidant (17 mg) was added. A solution of NH_4Cl (11.5 g, 0.21 mole) in water (80 ml) was added dropwise over 30 minutes under a nitrogen atmosphere. The black mixture was separated and the aqueous portion washed with ether (60 ml). The organic extracts were combined, dried over $MgSO_4$, and evaporated in

vacuo to give a brown oil (3.2 g, 91%). TLC (ether-pentane, 15:85), R_f 0.28; IR 3370 (OH), 3250 (HC≡C-); NMR δ 7.2-5.0 (7H, m, olefin, HC≡C), 2.60 (1H, s, OH), 1.93 (3H, s, 7-CH_3), 1.60 (3H, s, 3-CH_3).

2-^{14}C,2,6-Trimethylcyclohexanone (12)

A solution of the potassium enolate of 2,6-dimethylcyclohexanone was prepared by addition of an equivalent of the ketone to an equivalent of potassium hydride (24% in oil suspension) in tetrahydrofuran[3]. The mixture was stirred for 30 minutes and an aliquot was titrated by reaction with methyl iodide followed by gas chromatographic analysis. The tetrahydrofuran solution (12.6 ml, 6.67 mmoles of the enolate) was cooled in liquid nitrogen and methyl-^{14}C iodide (6.67 mmoles, 5.0 mCi/mmole) was added. The cooling bath was removed and the mixture was stirred at ambient temperature for 40 minutes. The mixture was treated with 70% tetrahydrofuran (1 ml) whereupon the solvent was removed under a stream of argon. The aqueous residue was extracted twice with 10-ml portions of ether, followed by washing of the extract with 1 ml of water. The extract was dried over $MgSO_4$ and evaporated under an argon stream to leave a pale yellow oil. Gas chromatographic analysis indicated product (90%), 2,6-dimethylcyclohexanone (8%) and tetramethylketone (2%).

9-(1'-Hydroxy-2'-^{14}C,2',6'-trimethylcyclohexyl)-3,7-dimethyl-2,4,6-nonatrien-8-yn-1-ol (7)

An ether solution of the triene-1-yn-3-ol (5) was stirred with 0.11N H_2SO_4 for 16 hours to afford the triene-8-yn-1-ol (6) by the procedure of Jones and Evans[4]. The alkynol (6, 1.27 g, 7.85 mmoles) in benzene (6 ml) was added to ethylmagnesium bromide (15.7 mmoles) in

ether (7 ml) and the mixture was stirred at reflux for 1 hour. The trimethylcyclohexanone-^{14}C (12, 6.67 mmoles) in ether (5 ml) was added over 15 minutes and the mixture was stirred at reflux for 1 hour. Saturated NH_4Cl (11 ml) was then added and the mixture stirred for a few minutes. After separation of the organic phase, the aqueous portion was extracted with ether (10 ml). The combined organic extracts were dried over $MgSO_4$ and evaporated under an argon stream to afford 2.0 g of crude product. TLC (10% MeOH/$CHCl_3$) showed approximately 90% of the material at R_f 0.58; m.p. 130-133° (reported[1] 122-127°), UV, λ_{max} (CH_3OH) 302, 317 nm in agreement with the literature[1,4].

Retinyl Acetate (10)

To a stirred mixture of lithium aluminum hydride (0.51 g, 13.3 mmoles) and ether (10 ml) was added a solution of the yn-ol (7) in ether (100 ml) over 30 minutes. The mixture was stirred at reflux for 3 hours and cooled in an ice bath. Water (3 ml) was cautiously added and the ether solution decanted from the precipitated aluminum salts. The residue was extracted twice more with 10-ml portions of ether. The total ether extract was dried over $MgSO_4$ and stabilized by addition of BHT (50 mg). TLC on an aliquot showed 80% of the radioactivity in a spot at R_f 0.65 and corresponding to the tetrene (8). IR showed loss of the 2100 cm^{-1} band in 7 assigned to the acetylenic moiety; UV λ_{max} (EtOH) 295, 309, 323 nm corresponded to the literature spectrum[1]. In a cold run 8 was obtained in 77% yield with spectral properties equal to those reported.

The ethereal solution of 8 was concentrated to a volume of 50 ml under a stream of argon and, with protection from light, treated with pyridine (3.7 ml). The mixture was cooled to 0-5° and a solution of

acetyl chloride (1.2 g, 15.3 mmoles) in ether (50 ml) was added over 30 minutes with maintenance of the temperature below 10° C. The mixture was kept for 40 minutes at ambient temperature and treated with 1N H_2SO_4 (25 ml), keeping the temperature at 5-10°C. After separation, the ether solution was dried over $MgSO_4$ and concentrated to about 5 ml under an argon stream. An aliquot showed acetate carbonyl at 1720 cm^{-1} in the IR. The crude acetate (9) was dissolved in 350 ml of toluene containing p-toluenesulfonic acid (15 mg). The solution was heated at 85°C under an argon atmosphere for 30 minutes, cooled to room temperature and washed with saturated $NaHCO_3$ (20 ml); TLC (ether-hexane, 1:9) showed several spots with 15% of the radioactivity contained in the spot, R_f 0.4, corresponding to authentic retinyl acetate. Preparative thin layer chromatography of an aliquot gave chromatographically pure material corresponding to a 5% yield from the labeled trimethylcyclohexanone (12). Cold runs similarly averaged 10-15% yields.

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