THE SYNTHESIS OF 13-CIS-RETINOIC ACID-6,7-^{14}C

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SUMMARY

A route to crystalline 13-cis-retinoic acid-6,7-^{14}C (1) has been developed. Addition of acetylene-1,2-^{14}C to 6-methyl-5-hepten-2-one (4) gave dehydrolinalool-1,2-^{14}C (5) which was catalytically transformed into citral-1,2-^{14}C (6) and then condensed with acetone to give pseudoionone-4,5-^{14}C (7). Ring closure of 7 gave β-ionone-6,7-^{14}C (8) which upon addition of vinyl Grignard gave vinyl-β-ionol-6,7-^{14}C (9). Conversion of 9 to its triphenylphosphonium salt 10 and condensation of the Wittig reagent from 10 with 4-hydroxy-3-methylbut-2-enolide (11) gave a mixture from which pure 13-cis-retinoic acid-6,7-^{14}C (1) could be crystallized.

Key Words: 13-cis-Retinoic acid-6,7-^{14}C, ^{14}C-ring labelled 13-cis-Retinoic acid, 11-cis, 13-cis-Retinoic acid-6,7-^{14}C.

Studies to determine the tissue distribution and metabolism of 13-cis-retinoic acid (1), which has generated interest as a potential cancer prophylactic agent,[1] required the preparation of this synthetic retinoid labelled with ^{14}C. Side chain degradation, observed[2-4] in the metabolism of trans -retinoic acid (2), necessitated that the ^{14}C label be located in or adjacent to the ring in 1. Most syntheses directed at the preparation of retinoic acid produce the thermodynamically preferred trans isomer (2) as the major product.[5] Through the use of a procedure[6,7] which permits the syntheses of polyenes having cis-stereochemistry, compound 1 was prepared and isolated in a purified crystalline state. We now report the preparation of the isotopically labelled analogue, 13-cis retinoic acid-6,7-^{14}C (1).

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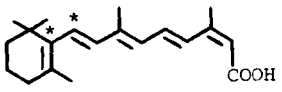
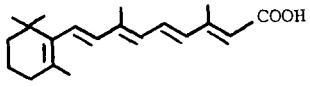


1, 13-cis-retinoic acid-6,7-^{14}C



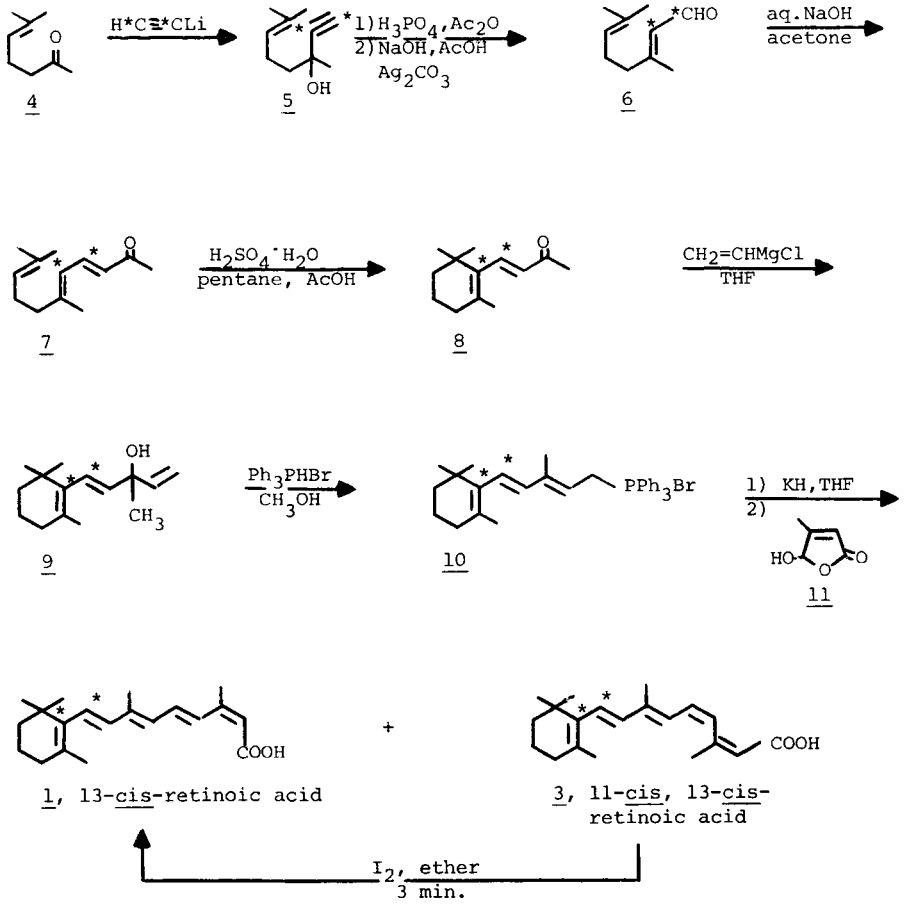
2, trans-retinoic acid

Synthesis of the 13-cis-retinoic acid (1) has been reported[6] by the reaction of the Wittig reagent generated from β-ionylideneethyl triphenylphosphonium bromide (10) with the lactone, 4-hydroxy-3-methyl-but-2-enolide (11, scheme 1). The crude product, which consists of a mixture of the 13-cis-isomer (1) and the 11-cis, 13-cis-isomer (3) can be further enriched in 1 by equilibration with iodine. Access to ring labelled precursors to 10 was readily achieved by the pathway outlined in Scheme 1. Thus acetylene-1,2-^{14}C[8] was converted to its lithium derivative in tetrahydrofuran and condensed[9] with 6-methyl-5-hepten-2-one (4) to give dehydrolinalool-1,2-^{14}C (5). Silver catalyzed rearrangement[10] of 5 gave citral-1,2-^{14}C (6) which was condensed with acetone[11] to give pseudoionone-4,5-^{14}C (7). Treatment of 7 with sulfuric acid monohydrate in acetic acid[12] gave β-ionone-6,7-^{14}C [4-(2,6,6-trimethyl-1-cyclohexen-1-yl-1-^{14}C)-3-buten-2-one-4-^{14}C, 8] which upon addition of vinyl magnesium chloride[13] yielded vinyl β-ionol-6,7-^{14}C [5-(2,6,6-trimethyl-1-cyclohexen-1-yl-1-^{14}C)-3-methyl-1,4-pentadien-3-ol-5-^{14}C, 9]. Treatment of 9 with triphenylphosphonium bromide[14] gave the phosphonium salt[15] 10.

To conserve the radiochemical yield, 10 was converted to the Wittig reagent using two equivalents of potassium hydride in tetrahydrofuran. Upon addition of lactone 11 to the reaction mixture, the second equivalent of potassium hydride serves to remove the active hydrogen from the acetal, thereby making a two-fold excess[6] of the Wittig reagent unnecessary. Under these conditions, the product isolated (62% yield by weight) consisted of a mixture of the 13-cis isomer

(1) and the 11-cis, 13-cis isomer (3) in the ratio of 6:4, respectively (as determined by nmr). Equilibration of the mixture with iodine in ether for 3 minutes gave 82% of 1, 12% of 3 and 6% of the all trans isomer 2. Upon addition of carrier 1, this mixture gave pure 13-cis-retinoic acid-6,7-^{14}C (1) upon crystallization from ethanol.

Scheme 1



EXPERIMENTAL

General. All reactions were carried out under a nitrogen or argon atmosphere in amber glassware. All solvents were distilled (tetrahydrofuran was distilled from sodium ribbon using benzophenone as an indicator) and magnesium sulfate was used as the drying agent. Spectra were recorded on standard instruments by the staff of the Physical Chemistry Department of Hoffmann-La Roche Inc. Radiochemical purity was determined on thin-layer chromatograms with a Packard Model 7201 Radiochromatogram Scanner System and radioactivity was measured by the liquid scintillation technique (BBOT cocktail) with a Packard Tricarb Model 2010 spectrometer.

Dehydrolinalool-1,2-^{14}C (5). Tetrahydrofuran (25 mL) was titrated to dryness with butyllithium using triphenylmethane as an indicator (pink color). After additional butyllithium (1.0 mmol, 0.58 mL, 1.74 M) was added, the flask was frozen in liquid nitrogen and evacuated (1 micron). Acetylene-1,2-^{14}C[9] (0.92 mmol, 108.2 mCi, 117.6 mCi/mmol) was introduced by vacuum transfer, the flask sealed and the solution stirred at -60° for 1 hour. After being refrozen (liquid nitrogen), the flask was again evacuated and 6-methyl-5-hepten-2-one (4, 1 mmol, 126 mg, 147 µL, Aldrich) was introduced by vacuum transfer. The solution was stirred 3 hr at 0°, then overnight at room temperature.[8] At 0°, aqueous ammonium chloride was added, the solution stirred 10 min., then extracted with ether (3 x 50 mL). The combined ether extracts were dried, concentrated at atmospheric pressure (vigreaux column), and the residue distilled (80° bath temp., 14 mm) to yield 85 mg (0.56 mmol, 66 mCi, 61% yield) of 5.

β-Ionone-6,7-^{14}C[4-(2,6,6-trimethyl-1-cyclohexyl-1-yl-1-^{14}C)-3-buten-2-one 4-^{14}C 8]. Dehydrolinalool-1,2-^{14}C (5) (85 mg, 0.56 mmol, 66 mCi) was dissolved in acetic anhydride (1 mL) to which phosphoric acid (85%, 2 µL, 3.7 mg) was then added with stirring. The solution was kept overnight at room temperature under nitrogen. Upon addition of sodium hydroxide (30 mg), acetic acid (2 mL) and

silver carbonate (spatula tip,~25 mg, freshly prepared from silver nitrate and sodium carbonate) the solution was stirred at 90° for 1.5 hr.[10] After being cooled, the solution was diluted with brine and extracted with pentane (3 x 50 mL). The combined extracts were washed with aqueous sodium bicarbonate and brine, then dried and concentrated at atmospheric pressure. The crude residue of 6 was dissolved in acetone (15 mL), diluted with aqueous sodium hydroxide (500 mg/15 mL) and heated to 40° for 3 hr.[11] The solution was cooled, mixed with brine, and extracted with pentane (3x50 mL). The combined pentane layers were washed with brine, dried, and concentrated at atmospheric pressure. The crude residue in pentane (5 mL) was added to a mixture of sulfuric acid monohydrate (4 g) and acetic acid (1.1 mL) at -20° over a period of 4 min. with vigorous stirring.[12] Stirring was continued 10 min at 7° and then all at once, ice water (30 g) was added. The mixture was extracted with pentane (3x25 mL) and the combined organic extracts washed with water until acid free. After being dried and concentrated (atmospheric pressure), the crude residue was diluted with carrier β-ionone (120 mg, 129 μL) and distilled (150° bath temperature/0.5 mm) to yield 148 mg (0.77 mmol, 20.5 mCi, 26.6 mCi/mmol, 31% radiochemical yield from 5) of 8.

Vinyl β-ionol-6,7-^{14}C [5-(2,6,6-trimethyl-1-cyclohexen-1-yl-1-^{14}C-3-methyl-1,4-pentadien-3-ol-5-^{14}C, 9]. β-ionone-6,7-^{14}C (8, 148 mg, 0.77 mmol) in 25 ml of tetrahydrofuran was treated with vinyl magnesium chloride[13] (3.5 mmol, 1 mL, 3.5M) at 5°. Stirring was continued at this temperature for 30 min., then the reaction was quenched with saturated ammonium chloride in 5 mL of 0.6N of ammonium hydroxide. The slurry was filtered, taken up in hexane (2 mL) and applied to a column of basic alumina (15 g, 6% water). Elution with 20% ethyl acetate in hexane provided, after combination of the pure fractions, 103 mg (0.47 mmol, 12.5 mCi, 26.6 mCi/mmol, 61% yield) of 9.

13-cis-Retinoic acid-6,7-^{14}C. Vinyl β-ionol-6,7-^{14}C (103 mg, 0.47 mmol) was stirred with triphenylphosphine hydrobromide[14] (190 mg, 0.58 mmol) in methanol (10 mL) for two days at room temperature.[6] Concentration of the methanol solution in vacuo and extraction of the residue with ether (3x5 mL) gave 10[15] as a white solid which was dried under vacuum (249 mg, 0.47 mmol, 100%). The phosphonium salt (10) was slurried in 25 ml of dry tetrahydrofuran and treated with potassium hydride (1.0 mmol, 167 mg, 24% oil dispersion). After a dark red color formed, the solution was stirred twenty minutes at room temperature then cooled to 0° and 4-hydroxy-3-methylbut-2-enolide[6] (11, 57 mg, 0.5 mmol) in tetrahydrofuran (1 mL) was added. The reaction mixture was allowed to warm to room temperature and then stirred overnight. At 0°, the dark reaction mixture was quenched with ice water (60 mL) and the resulting alkaline solution extracted with ether (4x50 mL) to remove neutral materials. The combined ether extracts were washed once with water (30 mL) and the combined aqueous layers acidified to pH 4 with sulfuric acid (2N). The product was extracted with ether (3x50 mL) and the combined extracts washed with water and brine, then dried and concentrated under vacuum to give 87 mg of a mixture of 1 and 3 as an orange oil.

A similar experiment carried out with unlabelled material gave a mixture which was analyzed by nmr and showed 62% of the 13-cis isomer (1, δ 2.09, 13-methyl)[6] and 38% of the 11-cis, 13-cis isomer (3, δ 2.20, 13-methyl).[6] After equilibration of the mixture with iodine in ether (3 min., room temperature, aqueous sodium thiosulfate quench), the nmr spectrum showed 82% of 1, 12% of 3 and 6% of the trans-isomer (2, δ 2.37, 13-methyl).[6]

Equilibration of the ^{14}C labelled mixture of 1 and 3 with iodine as described above gave 85 mg (7.5 mCi) of orange oil which was diluted with an equivalent weight of non-labelled 1 and crystallized from 1.5 mL of ethanol.

The first crop obtained, contained 1.59 mCi of activity; an additional 50 mg of carrier 1 was added to the mother liquor (with warming) to precipitate an additional 1.09 mCi of activity. On standing the mother liquor deposited an additional 1.16 mCi of orange crystals. Analysis of the three crops of material by tlc (silica gel, 25% ethyl acetate/hexane) indicated the purity to be 80-90% of 1 with the chief contaminant being 2. The three crops were combined and recrystallized from 2 mL of ethanol to give 109 mg (1.1 mCi, 10.1 μCi/mg, 3.02 mCi/mmol, 9% yield from 9, 1% from acetylene-1,2-^{14}C) of 1 having a purity of >98% by tlc.

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